Medical Marijuana Program

165 Capitol Avenue, Room 145, Hartford, CT 06106-1630 • (860) 713-6066
E-mail: dcp.mmp@ct.gov • **Website:** www.ct.gov/dcp/mmp

Petition to Add a Medical Condition, Medical Treatment or Disease to the List of Debilitating Conditions

INSTRUCTIONS: Please complete each section of this Petition and attach all supportive documents. All attachments must include a title referencing the Section letter to which it responds. Any Petition that is not fully or properly completed will not be submitted to the Board of Physicians.

Please Note: Any individually identifiable health information contained in a Petition shall be confidential and shall not be subject to disclosure under the Freedom of Information Act, as defined in section 1-200, Connecticut General Statutes.

Section A: Petitioner's Information

Name (First, Middle, Last): [redacted]			
Home Address (including Apartment or Suite #): [redacted]			
City: [redacted]		State: [redacted]	Zip Code: [redacted]
Telephone Number:	E-mail Address: [redacted]		

Section B: Medical Condition, Medical Treatment or Disease

Please specify the medical condition, medical treatment or disease that you are seeking to add to the list of debilitating medical conditions under the Act. Be as precise as possible in identifying the condition, treatment or disease.

Severe Psoriasis and Psoriatic Arthritis

Section C: Background

Provide information evidencing the extent to which the condition, treatment or disease is generally accepted by the medical community and other experts as a valid, existing medical condition, medical treatment or disease.

- Attach a comprehensive definition from a recognized medical source.
- Attach additional pages as needed.

A chronic, inflammatory, painful, disfiguring and disabling disease with no cure. 30% will also be diagnosed with psoriatic arthritis that is painful debilitating and causes joint damage (See attached #'s1,2,3)

Section D: Negative Effects of Current Treatment

If you claim a treatment, that has been prescribed for your condition causes you to suffer (i.e. severe or chronic pain, spasticity, etc.), provide information regarding the extent to which such treatment is generally accepted by the medical community and other experts as a valid treatment for your debilitating condition.

- Attach additional pages as necessary.
- If not applicable, please indicate N/A.

Most effective agents used to treat severe psoriasis carry high risk of significant morbidity including skin cancers, lymphoma and liver disease and NONE help the severe irritation pain of the pruritus (Attached #3,4,5, 12)

Section E: Negative Effects of Condition or Treatment

Provide information regarding the extent to which the condition or the treatments thereof cause severe or chronic pain, severe nausea, spasticity or otherwise substantially limits one or more major life activities.

- Attach additional pages as necessary.

See: (National Psoriasias Foundation Attachment #1) (American Academy of Dermatology Attachment # 2)

(Mayo Clinic Attachment # 3) (Wake Forest Baptist Medical Center Attachment # 6) (#7) (#8) (#9)

Section F: Conventional Therapies

Provide information regarding the availability of conventional medical therapies, other than those that cause suffering, to alleviate suffering caused by the condition or the treatment thereof.

- Attach additional pages as necessary.

All available therapy's carry significant morbidity risk including skin cancers, lymphoma and such. That scares the

life out of me and such options cannot for me.(13) No therapy specifically targets severe pruritus suffering (#12,13)

Section G: General Evidence of Support for Medical Marijuana Treatment

Provide evidence, generally accepted among the medical community and other experts, that supports a finding that the use of marijuana alleviates suffering caused by the condition or the treatment thereof.

- Attach additional pages as necessary.

See Attachments; (#10), (#11), (13), (14), (15), (16),(18),(19), (20), (24)

Section H: Scientific Evidence of Support for Medical Marijuana Treatment

Provide any information or studies regarding any beneficial or adverse effects from the use of marijuana in patients with the condition, treatment or disease that is the subject of the petition.

- Supporting evidence needs to be from professionally recognized sources such as peer reviewed articles or professional journals.
- Attach complete copies of any article or reference, not abstracts.

See Attached: (#'s 21,22,23,24)

Section I: Professional Recommendations for Medical Marijuana Treatment

Attach letters in support of your petition from physicians or other licensed health care professionals knowledgeable about the condition, treatment or disease at issue.

See section J. I am already enrolled under a related condition

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Section J: Submission of Petition

In the event you are unable to answer or provide the required documentation to any of the Sections above (excluding Section D); provide a detailed explanation indicating what you believe is "good cause" for not doing so.

- Attach additional pages as necessary.

See Attachment: (#25)

I hereby certify that the above information is correct and complete.

My signature below attests that the information provided in this petition is true and that the attached documents are authentic. I formally request that the commissioner present my petition and all supporting evidence to the Board of Physicians for consideration.

Signature: [redacted]	Date Signed: 3-2-2014

#'s 1 & 2

psoriasis and psoriatic arthritis.

The National Psoriasis Foundation is the largest psoriasis patient advocacy organization and charitable funder of psoriatic disease research worldwide states psoriasis and psoriatic arthritis. Psoriasis, which affects 7.5 million Americans, is the most prevalent autoimmune disease in the nation. Psoriasis is a noncontagious, chronic, inflammatory, painful, disfiguring and disabling disease for which there is no cure. It is a systemic disease that shows on the skin, most often as red, scaly patches that itch and may bleed. stress is a documented trigger for flares of both psoriasis and psoriatic arthritis. Psoriasis requires sophisticated medical intervention. Psoriasis is a lifelong chronic disease and requires timely and appropriate medical care. A wide range of treatment options is available; however, adverse side effects and success vary from patient to patient. For many people with psoriasis, existing treatments are not effective. *See attachment #1*

American Academy of Dermatology Psoriasis (sore-EYE-ah-sis) is a medical condition that occurs when skin cells grow too quickly. Faulty signals in the immune system cause new skin cells to form in days rather than weeks. The body does not shed these excess skin cells, so the cells pile up on the surface of the skin and lesions form. For some people, psoriasis is a nuisance. Others find that psoriasis affects every aspect of their daily life. The unpredictable nature of psoriasis may be the reason. Psoriasis is a chronic (lifelong) medical condition. Some people have frequent flare-ups that occur weekly or monthly. Others have occasional flare-ups. When psoriasis flares, it can cause severe itching and pain. Sometimes the skin cracks and bleeds. When trying to sleep, cracking and bleeding skin can wake a person frequently and cause sleep deprivation. A lack of sleep can make it difficult to focus at school or work These cycles of flare-ups and remissions often lead to feelings of sadness, despair, guilt and anger as well as low self-esteem. Depression is higher in people who have psoriasis than in the general population. Feelings of embarrassment also are common. *See attachment #2*

#1

NATIONAL PSORIASIS FOUNDATION®

Psoriasis and Mental Health Issue Brief

Executive Summary

The National Psoriasis Foundation is the largest psoriasis patient advocacy organization and charitable funder of psoriatic disease research worldwide. The Foundation exists to find a cure for psoriasis and psoriatic arthritis. Psoriasis, which affects 7.5 million Americans, is the most prevalent autoimmune disease in the nation. Psoriasis is a noncontagious, chronic, inflammatory, painful, disfiguring and disabling disease for which there is no cure. It is a systemic disease that shows on the skin, most often as red, scaly patches that itch and may bleed. Psoriasis requires sophisticated medical intervention.

People with psoriasis experience higher rates of depression and anxiety, and people with severe psoriasis die, on average, four years younger than people without the disease. As a systemic, inflammatory autoimmune disease, psoriasis is also connected with an elevated risk for other serious, chronic and/or life-threatening conditions, including cardiovascular disease, diabetes, stroke and cancer. As many as 30 percent of people with psoriasis will be diagnosed with psoriatic arthritis, a specific form of arthritis that is painful and debilitating and causes joint damage.

Psoriasis is a lifelong chronic disease and requires timely and appropriate medical care. A wide range of treatment options is available, however, adverse side effects and success vary from patient to patient. For many people with psoriasis, existing treatments are not effective, appropriate or may not be accessible due to cost and insurance barriers. Further, the economic consequences of psoriasis, both for individuals and the health care system, are significant. In 2008, the annual cost of psoriasis in the U.S. was estimated at $11.25 billion. People with psoriasis have significantly higher health care resource utilization and costs than the general population. As such, the National Psoriasis Foundation maintains a strong commitment to engaging in efforts to secure federal funding for public policies and programs to achieve a cure, make progress toward better treatments, and end health insurance policies and procedures that are harmful to people with psoriasis and psoriatic arthritis.

In addition to the physical impact, psoriasis significantly affects mental and emotional functioning:

- Psoriasis is independently associated with depression and the risk of psychiatric comorbidity increases with the severity of psoriasis.
- People with psoriasis are twice as likely to have thoughts of suicide compared to the general population and people with other chronic diseases. Almost ten percent of psoriasis patients surveyed reported a wish to be dead.
- Psoriasis is independently associated with stress-related and behavioral disorders.
- Women, minorities and young people experience greater social and emotional impacts due to their psoriasis and psoriatic arthritis, as compared to the general psoriasis population.
- More than 80 percent of all patients surveyed report their disease to be a moderate or large problem in their everyday life.

With the significant relationships between psoriasis and other disabling, life-threatening and costly chronic conditions, the National Psoriasis Foundation urges greater collaborative federal investment in—and commitment to—leveraging existing funding and knowledge, expanding the base of understanding of the disease and engaging other stakeholders to contribute to psoriasis research efforts that benefit both psoriasis and conditions comorbid with this difficult disease, including mental health issues associated with psoriasis such as depression, suicidality, anxiety and other stress-related and behavioral disorders.

In fiscal year (FY) 2013, the Foundation urges additional attention to—and funding of research into—the serious mental health implications of psoriasis. The Foundation calls on Congress to encourage a process within National Institutes of Health (NIH), including the National Institute of Mental Health (NIMH), to guide now disparate research efforts in order to optimize federal research investments and ensure discoveries are applied across common goals that advance the understanding of causes, treatments and cures for psoriasis and its comorbid conditions.

Psoriasis and Mental Health Issue Brief

The National Psoriasis Foundation, the largest psoriasis patient advocacy organization and charitable funder of psoriatic disease research worldwide, assists approximately 1.5 million people annually through educational programs and services. Psoriasis is a noncontagious, chronic, inflammatory, painful, disfiguring and disabling autoimmune disease for which there is no cure. It appears on the skin, most often as red, scaly patches that itch and may bleed and it requires sophisticated medical care. As a systemic, inflammatory autoimmune disease, psoriasis is connected with an elevated risk for other serious, chronic and life-threatening conditions, including cardiovascular disease, diabetes, stroke and cancer. Current studies indicate that the prevalence of psoriasis in the United States ranges between two and three percent, affecting approximately 7.5 million Americans.[1]

Research has established that psoriasis is associated with mental health disorders and carries an increased risk of psychiatric comorbidities, including depression, anxiety and suicidality.* Furthermore, psoriasis is associated with significant adverse impacts to psychosocial functioning and emotional health. As such, people with psoriasis need access to comprehensive, quality and affordable care to address the mental health aspects of living with the disease, as well as the physical needs and challenges that the disease poses for patients.

The National Psoriasis Foundation is concerned that despite some recent breakthroughs, too many people with psoriasis do not have access to the medical care and the appropriate treatment that they need either for their psoriasis or for the mental health issues they may face as a result of their psoriasis. As such, the National Psoriasis Foundation maintains a strong commitment to engaging in efforts to secure federal funding for public policies and programs to achieve a cure, make progress toward better treatments and end health insurance policies and procedures that are harmful to people with psoriasis and psoriatic arthritis. We must ensure that people with psoriatic disease have full access to the care and treatment options they need to function normally and live full lives.

The Chronic, Serious, Life-Altering and Costly Impact of Psoriasis

Psoriasis requires steadfast treatment and lifelong attention, especially since it most often strikes between age 15 and 25. Unfortunately, psoriasis often is overlooked or dismissed because it is not typically a direct cause of death. It is commonly and incorrectly considered by insurers, employers, policymakers and the public as a mere annoyance—a superficial "skin problem," mistakenly thought to be contagious and/or due to poor hygiene. As such, treatment for it may be categorized—wrongly—as "cosmetic" and "not medically necessary." Lack of appropriate treatment for psoriasis can result in serious adverse impacts to functioning, including loss of mobility, pain, isolation and depression, and may contribute to comorbid conditions.

While on its own psoriasis can be a painful, and physically and psychologically devastating condition, it often is accompanied by many other serious issues.[2]

- Up to 30 percent of people with psoriasis also develop psoriatic arthritis, which causes pain, swelling and stiffness around the joints. People with mild psoriasis are just as likely to develop psoriatic arthritis as those with moderate or severe forms of the disease—in fact, arthritis precedes skin involvement in some patients.
- Psoriasis is associated with a significant increased risk for cardiovascular disease and myocardial infarction (heart attack) that is independent of other comorbidities and may be related to age and disease severity.[3]
- People with psoriasis have an increased risk of diabetes[4], hypertension[5], and stroke[6] independent of other risk factors. A higher prevalence of atherosclerosis[7], Crohn's disease[8], cancer[9], metabolic syndrome[10], obesity[11] and liver disease[12] are found in people with psoriasis as compared to the general population. In addition a connection between psoriasis and multiple sclerosis has been reported.[13]†
- Studies have shown that psoriasis causes as much mental and physical disability as other major chronic diseases.[14] Other recent studies have established that the risk of premature death is 50 percent higher for people

* Suicidality includes suicidal thoughts, suicide attempts and completed suicides.
† For more information about comorbidities, see the National Psoriasis Foundation Comorbid Conditions Issue Brief.

with severe psoriasis and these individuals die, on average, four years younger than those without psoriasis.[15]

- In 2008, the total cost of psoriasis in the U.S. was estimated at $11.25 billion due to medical expenses and lost wages.[16]
- People with severe psoriasis are significantly more likely to be considered "low-income" than those with mild disease and to report that psoriasis is the reason they are not working.[17]

The Psychosocial Impact of Psoriasis

In addition to the physical impact, psoriasis can exact a significant emotional toll. People with psoriasis report feeling self-conscious, embarrassed and helpless. The physical pain and seriousness of the disease, as well as its adverse emotional effects, can lead to a cycle of despair. Misperceptions about the nature and cause of the disease and the associated social stigma contribute to poor psychosocial adjustment and low self-esteem. These problems are compounded by inadequate access to treatments and the fact that there is no cure, which can lead to depression and anxiety. This cycle is continued when unaddressed mental health problems prevent patients from effectively managing their disease. People cope with psoriasis in a variety of ways, but commonly rely on mechanisms such as avoiding being in public, abusing alcohol and overeating. These coping mechanisms can contribute to or exacerbate other serious problems, such as comorbid health conditions associated with psoriasis including heart disease[5], diabetes[6], obesity[12] and smoking[18]. These psycho-social impacts in turn can negatively influence the progression of the disease, as stress is a documented trigger for flares of both psoriasis and psoriatic arthritis.

Research on the association between psoriasis and mental health disorders has established:

- Patients with psoriasis have a 39 percent increased risk of depression, a 31 percent increased risk of anxiety and a 44 percent increased risk of suicidality. Patients with severe psoriasis have a 72 percent increased risk of depression.[19]
- The prevalence of depression in patients with moderate to severe psoriasis is approximately 24 percent[20], as compared to an estimated 6.7 percent of the adult U.S. population.[21]
- As many as 60 percent of psoriasis patients report clinically significant psychiatric symptoms (such as depression) and may receive a psychiatric diagnosis.[22]
- Patients with psoriasis and psoriatic arthritis experience fatigue and sleep impairment, linked to factors including pruitus (itch), depression, pain and obstructive sleep apnea.[23]

Psoriasis is also linked to increased risk of suicidality[24]:

- It has been documented that individuals with psoriasis are twice as likely to have thoughts of suicide as people without psoriasis or with other chronic conditions.[25]
- Almost ten percent of psoriasis patients surveyed report a wish to be dead and five percent report actively thinking about suicide.[26]

With respect to stress and psoriasis, studies have found that:

- Psoriasis is independently associated with stress-related disorders and behavior disorders.[27]
- Psoriasis with onset prior to age 40 is associated with greater difficulties in self-assertion and expression of anger, a personality trait that may adversely affect the patient's capacity to cope with stress.[28]
- Post Traumatic Stress Disorder (PTSD) is associated with psoriasis and other immune-mediated diseases.[29]
- A patient's psychological state can adversely impact treatment compliance and coping strategies[30], and female gender is predictive of higher stress levels due to psoriasis[31].
- Psoriasis has a significant impact on other family members. Ninety-two percent of relatives/partners of those

with psoriasis indicate that the patient's psoriasis has impacted their quality of life.[32]

People with psoriasis report that their disease can also cause severe disruptions in their everyday interactions with colleagues, friends and family, as well as in their close personal or intimate relationships. In children, social development, which contributes to many developmental milestones, is particularly impaired.[33]

National Psoriasis Foundation patient survey panels[34] consistently capture the emotional toll psoriasis takes on patients:

- More than 80 percent of patients report their disease to be a moderate or large problem in their everyday life.
- Twenty-five percent of people with psoriasis and almost half of people with psoriatic arthritis report that their disease negatively affects their job.
- Nearly one-third report that their psoriasis interferes with their sexual activities.
- More than 40 percent of people with psoriasis report experiencing social discrimination and humiliation (e.g. being refused service at hair or nail salons, not being permitted to use public pools, gyms or health clubs), which contributes to feelings of shame and isolation.
- Survey results indicate that women, minorities and young people experience greater social and emotional impacts due to their psoriasis and psoriatic arthritis, as compared to the general psoriasis population.

Ways to Better Address the Mental Health Aspects of Psoriasis

The adverse mental health aspects of psoriasis not only have a direct psychological impact, but also potentially worsen the disease, which in turn compounds the psychosocial effects. A patient's mental health state can interfere with their ability to adhere to and respond to treatment. Moreover, control of psoriasis symptoms is associated with improvements in psychological symptoms.[35] As such, people with psoriasis must receive comprehensive treatment, including primary, specialty and psychological care. In addition, the development of quality measures and standards of care related to holistically treating psoriasis patients would help improve care delivery and patient well-being and outcomes. Studies testing "bio-psychosocial"[‡] interventions to improve psoriasis and address the negative psychosocial impacts would provide significant advances in care. This body of knowledge would lead to the development of programs to help patients adjust better and adhere to treatment regimens; it also would assist dermatologists and rheumatologists in more effectively screening their psoriasis patients for the mental health aspects of the disease. As patients with psoriasis have an increased risk of depression, anxiety and suicidality, it is important for clinicians to evaluate patients for these conditions in order to improve outcomes. [36]

Additional research is needed to study the behavioral, social and mental health effects of psoriasis. Specifically, National Psoriasis Foundation clinical advisors recommend that the federal government fund efforts to:

- Develop instruments to measure the mental health impact of psoriasis and/or to validate those already available for this disease;
- Identify any underlying biological mechanisms linking depression or other mental health states with psoriasis (e.g., is there a connection to inflammation?);
- Develop pharmacological and non-pharmacological treatments for co-occurring medical and psychological problems such as depression, anxiety and obesity;
- Further explore how negative social and psychological effects impact psoriasis (e.g., does the psychological component worsen the psoriasis?);
- Identify how best to help patients adhere to complex and sometimes unpleasant treatments; and

‡ "Bio-psychosocial" refers to looking at the mind, body and environment of a patient as equally important systems that are interlinked.

- Learn how to help patients cope with the common social challenges (e.g., stigmatization) resulting from psoriasis.

Conclusion

Given the significant and growing link between psoriasis and myriad other disabling, life-threatening and costly chronic conditions, including mental health issues such as depression, suicidality, anxiety and other stress-related and behavioral disorders, the National Psoriasis Foundation urges additional attention to—and funding of—collaborative interdisciplinary research into the connection between psoriasis and mental health. Such an increased investment and focused research will advance understanding of the risks posed to people with psoriasis, identify ways to address risks for these comorbidities as early as possible and improve treatment and outcomes for people living with psoriasis.

The National Psoriasis Foundation has serious concerns about the severe adverse impact that psoriasis has on the mental health status and well-being of people living with the disease. As such, in FY 2013 the National Psoriasis Foundation urges Congress to:

- Implement the CDC's psoriasis and psoriatic arthritis research agenda; and
- Encourage a process within NIH, including the National Institute of Mental Health (NIMH), to guide now disparate research efforts in order to optimize federal research investments and ensure discoveries are applied across common goals that complement and advance the understanding of causes, treatments and cures for both psoriasis and its comorbid conditions; and
- Encourage federal agencies to study the mental health aspects of psoriasis, identify appropriate interventions and make recommendations for providing physical and psychological care to people living with psoriasis.

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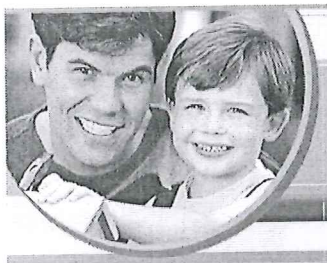

PsoriasisNet

A comprehensive online psoriasis information resource.

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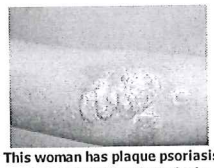
What is Psoriasis?

Psoriasis (sore-EYE-ah-sis) is a medical condition that occurs when skin cells grow too quickly. Faulty signals in the immune system cause new skin cells to form in days rather than weeks. The body does not shed these excess skin cells, so the cells pile up on the surface of the skin and lesions form.



This woman has plaque psoriasis, the most common type of psoriasis.

(Photos used with permission of the American Academy of Dermatology National Library of Dermatologic Teaching Slides)

What are the signs and symptoms?

The lesions vary in appearance with the type of psoriasis. There are five types of psoriasis: Plaque, guttate, pustular, inverse, and erythrodermic. About 80% of people living with psoriasis have plaque (plak) psoriasis, also called "psoriasis vulgaris." Plaque psoriasis causes patches of thick, scaly skin that may be white, silvery, or red. Called plaques (plax), these patches can develop anywhere on the skin. The most common areas to find plaques are the elbows, knees, lower back, and scalp.

Psoriasis also can affect the nails. About 50% of people who develop psoriasis see changes in their fingernails and/or toenails. If the nails begin to pull away from the nail bed or develop pitting, ridges, or a yellowish-orange color, this could be a sign of psoriatic (sore-EE-at-ic) arthritis. Without treatment, psoriatic arthritis can progress and become debilitating. It is important to see a dermatologist if nail changes begin or joint pain develops. Early treatment can prevent joint deterioration.

What causes psoriasis?

Psoriasis is not contagious. You cannot get psoriasis from touching someone who has psoriasis, swimming in the same pool, or even intimate contact. Psoriasis is much more complex.

So complex, in fact, scientists are still studying what happens when psoriasis develops. We know that the person's immune system and genes play key roles. In studying the immune system, scientists discovered that when a person has psoriasis, the T cells (a type of white blood cell that fights unwanted invaders such as bacteria and viruses) mistakenly trigger a reaction in the skin cells. This is why you may hear psoriasis referred to as a "T cell-mediated disease."

This reaction activates a series of events, causing new skin cells to form in days rather than weeks. The reason T cells trigger this reaction seems to lie in our DNA. People who develop psoriasis inherit genes that cause psoriasis. Unlike some autoimmune conditions, it appears that many genes are involved in psoriasis.

Scientists are still trying to identify all of the genes involved. One of the genes that has been identified is called PSORS1 (SORE-ESS-1). This is one of several genes that regulates how the immune system fights infection.

Scientists also have learned that not everyone who inherits genes for psoriasis gets psoriasis. For psoriasis to appear, it seems that a person must inherit the "right" mix of genes and be exposed to a trigger. Some common triggers are a stressful life event, skin injury, and having strep throat. Many people say that that their psoriasis first appeared after experiencing one of these. Triggers are not universal. What triggers psoriasis in one person may not cause psoriasis to develop in another.

Who gets psoriasis?

People worldwide develop psoriasis. In the United States, nearly 7.5 million people have psoriasis and about 150,000 new cases are diagnosed each year. Studies indicate that psoriasis develops about equally in males and females. Research also shows that Caucasians develop psoriasis more frequently than other races. A study conducted in the United States found the prevalence was 2.5% in Caucasians and 1.3% in African Americans.

A family history of psoriasis seems to increase the risk of developing psoriasis. It is important to know that a family history of psoriasis does not guarantee that someone will develop psoriasis.

When do people get psoriasis?

Psoriasis can begin at any age, from infancy through the golden years. There are, however, times when psoriasis is most likely to develop. Most people first

see psoriasis between 15 and 30 years of age. About 75% develop psoriasis before they turn 40. Another common time for psoriasis to begin is between 50 and 60 years of age.

Does psoriasis affect quality of life?

For some people, psoriasis is a nuisance. Others find that psoriasis affects every aspect of their daily life. The unpredictable nature of psoriasis may be the reason. Psoriasis is a chronic (lifelong) medical condition. Some people have frequent flare-ups that occur weekly or monthly. Others have occasional flare-ups.

When psoriasis flares, it can cause severe itching and pain. Sometimes the skin cracks and bleeds. When trying to sleep, cracking and bleeding skin can wake a person frequently and cause sleep deprivation. A lack of sleep can make it difficult to focus at school or work. Sometimes a flare-up requires a visit to a dermatologist for additional treatment. Time must be taken from school or work to visit the doctor and get treatment.

These cycles of flare-ups and remissions often lead to feelings of sadness, despair, guilt and anger as well as low self-esteem. Depression is higher in people who have psoriasis than in the general population. Feelings of embarrassment also are common.

Knowledge is power

As psoriasis is a life-long condition, it is important to take an active role in managing it. Learning more about psoriasis, seeing a dermatologist to discuss treatment options, and developing a healthy lifestyle can help people live life to the fullest.

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For an overview, visit the AAD pamphlet Psoriasis and Psoriatic Arthritis.

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#3

SECTION B,C and E ATTACHMENT

Itchy skin (pruritus)

By Mayo Clinic staff

Itchy skin is an uncomfortable, irritating sensation that can make scratching irresistible. With psoriasis, the life cycle of skin cells speeds up, resulting in a rapid buildup of rough, dead skin cells. These skin cells accumulate, forming thick silvery scales and itchy, dry, red patches that are sometimes painful extremely severe and uncomfortable and distracts you from your daily routines or prevents you from sleeping

When to see a doctor

See your doctor or consult a specialist in skin diseases (dermatologist) if the itching:

- Lasts more than two weeks and doesn't improve with self-care measures
- Is extremely severe and uncomfortable and distracts you from your daily routines or prevents you from sleeping
- Can't be easily explained
- Affects your whole body
- Is accompanied by other symptoms, such as extreme tiredness, weight loss, changes in bowel habits or urinary frequency, fever, or redness of the skin

Causes

Dry skin

Itchy skin that isn't accompanied by other obvious skin changes, such as a rash, is most often caused by dry skin (xerosis). Dry skin usually results from environmental factors that you can influence. These include hot or cold weather with low humidity levels, long-term use of air conditioning or central heating, and washing or bathing too much.

Other possible causes

Other conditions such as skin disorders, internal diseases, allergies and drug reactions can also cause itchy skin.

- **Skin conditions and rashes.** Many skin conditions cause itchy skin, including eczema (dermatitis), psoriasis, scabies, lice, chickenpox, hives and dermatographism. In these cases, the itching usually affects specific areas and is accompanied by other signs, such as red, irritated skin or bumps and blisters.
- **Internal diseases.** These include liver disease, malabsorption of wheat (celiac disease), kidney failure, iron deficiency anemia, thyroid problems and cancers, including leukemia and lymphoma. In these cases, the itching usually affects the whole body, rather than one specific area. The skin may look otherwise normal except for the repeatedly scratched areas.
- **Nerve disorders.** Conditions that affect the nervous system — such as multiple sclerosis, diabetes mellitus, pinched nerves and shingles (herpes zoster) — can cause itching.
- **Irritation and allergic reactions.** Wool, chemicals, soaps and other substances can irritate the skin and cause itching. Sometimes the substance causes an allergic reaction, such as in the case of poison ivy or cosmetics. Food allergies also may cause itchy skin reactions.
- **Drugs.** Reactions to drugs, such as antibiotics, antifungal drugs or narcotic pain medications, can cause widespread rashes and itching.
- **Pregnancy.** Some women experience itchy skin during pregnancy, especially on the abdomen, thighs, breasts and arms. Also, itchy skin conditions, such as dermatitis, can worsen during pregnancy.

Complications

Prolonged itching and scratching may increase the intensity of the itch, possibly leading to neurodermatitis (lichen simplex chronicus). Neurodermatitis is a condition in which an area of skin that's frequently scratched becomes thick and leathery. The patches can be raw, red or darker than the rest of your skin. Persistent scratching can also lead to a bacterial skin infection and permanent scars or changes in skin color.

Preparing for your appointment

You're likely to start by seeing your family doctor or primary care doctor. However, in some cases when you call to set up an appointment, you may be referred to a specialist in skin diseases (dermatologist).

Because appointments can be brief and there's often a lot of ground to cover, it can help to be well prepared. Here are some tips to help you get ready for your appointment and what to expect from your doctor.

Related conditions

Through examination and tests, your doctor may determine that your itching is, in fact, a symptom of another skin condition. Related itchy skin conditions include:

- **Dermatitis.** Also called eczema, dermatitis is an inflammation of the skin. There are different types of dermatitis, and the disorder can have many causes and occur in many forms. The most common type of eczema is atopic dermatitis. Generally, dermatitis describes swollen, reddened and itchy skin.
- **Psoriasis.** With psoriasis, the life cycle of skin cells speeds up, resulting in a rapid buildup of rough, dead skin cells. These skin cells accumulate, forming thick silvery scales and itchy, dry, red patches that are sometimes painful.
- **Tinea infections.** Athlete's foot, ringworm of the body, ringworm of the scalp and jock itch are caused by a fungal infection that develops on the top layer of your skin. These infections often cause round, flat patches of itchy skin.
- **Hives.** Hives are raised, itchy red bumps of various sizes that appear and disappear on your skin. Allergic reactions to medications or foods can cause hives. Skin writing (dermatographism) is a condition where stroking the skin causes hive-like lesions to develop in the touched areas.
- **Lice.** Body lice, pubic lice and head lice are common causes of intense itching. Lice are tiny, wingless, parasitic insects that feed on your blood. The infestation, which is easily spread through close physical contact, can cause small, red bumps.
- **Scabies.** Scabies is caused by a tiny, eight-legged burrowing mite called Sarcoptes scabiei. The presence of the mite leads to intense itching in the area of its burrows. Scabies is contagious and can spread quickly through close physical contact.

Treatments and drugs

Once a cause is identified, treatments for itchy skin may include:

Medications

- **Corticosteroid creams.** Applied topically, these may control itching. Your doctor may recommend applying the medicated cream to affected areas, then covering these areas with damp cotton material that has been soaked in water or other solutions. The moisture in the wet dressings helps the skin absorb the cream.
- **Oral antihistamines.** These include oral antihistamines for allergies or hives and corticosteroid creams for itching from skin inflammation.

Treating the underlying disease

If an internal disease is found, whether it's kidney disease, iron deficiency or a thyroid problem, treating that disease often relieves the itch. Other itch-relief methods also may be recommended.

Light therapy (phototherapy)

Phototherapy involves exposing your skin to certain wavelengths of ultraviolet light. Multiple sessions are usually scheduled until the itching is under control.

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DS00847 April 14, 2011

#4

Writing in the Journal of Dermatological Science, Jonathan Wilkinson (Nottingham University) and Elizabeth Williamson (Reading University) say that cannabinoids have anti-inflammatory properties and can inhibit the growth of cancer cell lines.

University of Maryland Medical Center **Systemic Medications**

Systemic treatment uses various medications that affect the whole body, not just the skin. Many systemic drugs used for psoriasis are also used for other severe diseases, including autoimmune diseases (especially rheumatoid arthritis) and cancer.

Systemic treatments for psoriasis may be taken by mouth or injection. The medicines can have significant side effects and are generally reserved for severe psoriasis. Systemic medications approved for treating psoriasis include:

- Cyclosporine
- Methotrexate
- Retinoids
- Biologic Response Modifiers
- Psoralen

Methotrexate

Methotrexate (Rheumatrex) is a biologic drug that interferes with cell reproduction and has anti-inflammatory properties. It is a first-line, or primary, systemic drug used to treat adults with severe psoriasis.

The drug is taken weekly, not daily.

Side Effects. Many patients are able to tolerate methotrexate with few side effects. Possible side effects include:

- Anemia, usually causing no noticeable symptoms
- Headache
- Mild and slow hair loss that is reversible when the medication is stopped

- Increased likelihood of becoming sunburned
- Mouth sores
- Nausea, usually mild and improves over time
- Possible muscle aches
- Rash
- Vomiting (rare)

Many of these side effects are due to folic acid deficiency. Patients should ask their doctor if they should take folic acid supplements (generally recommended at 1 mg daily).

More serious, but relatively uncommon side effects include:

- Increased risk for infections, particularly shingles and pneumonia. Methotrexate suppresses the immune system. Patients with active infections should avoid this drug.
- Infertility, miscarriage, and birth defects. This drug should not be used during pregnancy, because it can cause miscarriages or birth defects. It may harm fertility in men.
- Kidney damage.
- Liver damage, most commonly in patients with existing liver problems. Regular monitoring for liver toxicity includes blood tests and sometimes liver biopsies. Patients who are properly monitored rarely have any permanent liver damage.
- Cough and shortness of breath. Risk factors for these side effects include diabetes, existing lung problems, protein in the urine, and the use of rheumatoid arthritis drugs of a type called DMARD.
- Severe anemia. Folic acid supplements can offset this effect.
- Toxic effects on bone marrow. This can cause reduced blood cell production.

Despite methotrexate's side effects, some experts view it as the best therapy for widespread plaque psoriasis. It may also be effective for some patients with generalized erythrodermic and pustular psoriasis.

Methotrexate appears to be effective in children, but more safety research is needed.

Drug Interactions. Many drugs interact with methotrexate, occasionally with harmful results. For example, the antibiotic trimethoprim-sulfamethoxazole increases the toxicity of methotrexate.

Taking nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin, ibuprofen, or naproxen at the same time as methotrexate may change the blood levels of methotrexate. Always talk with your doctor before taking these or any other medications in combinations.

People Who Should Avoid Methotrexate. Pregnant and nursing mothers should never take methotrexate because it increases the risk for severe, even fatal, birth defects and miscarriage. The drug should be

discontinued several months before the actual pregnancy. Methotrexate may also cause temporary impairment of fertility in men. Patients with Hepatitis B should not take methotrexate.

People with the following conditions are unlikely to be given methotrexate:

- Alcoholism
- Anemia or other blood abnormalities
- Kidney problems
- Liver problems (including hepatitis)
- Peptic ulcers
- Suppressed immune system

Oral Retinoids

Oral retinoids are vitamin A-related medications taken by mouth. This group of medicines is also a first-line treatment for adults with severe psoriasis. Oral retinoids used for psoriasis include acitretin (Soriatane) and isotretinoin (Accutane).

Side Effects. All retinoids have the same potentially serious toxicities, as do high doses of vitamin A. Side effects include:

- Bone and joint pain
- Bruising
- Depression and possible suicide risk (with isotretinoin)
- Eye problems, including blurred vision, cataracts, conjunctivitis, and a sudden deterioration in night vision
- Fatigue
- Headache
- Increased bone growth, particularly in the ankles, pelvic area, and knees
- Increased triglyceride levels
- Liver damage
- Nail problems
- Skin and mucus membrane problems, including dry nose, nosebleeds, dry eyes, chapped lips, thinning hair, dry or "sticky" feeling skin, and peeling of the palms and soles

In rare cases, retinoids, particularly isotretinoin, may cause a condition called benign intracranial hypertension (pseudotumor cerebri), which occurs in the brain. Symptoms include headache, nausea,

vomiting, and blurred vision. Patients experiencing these symptoms should call a doctor immediately and stop taking the drug.

Cyclosporine

Cyclosporine (Neoral, Sandimmune, SangCya) blocks certain immune factors and may be effective for all forms of psoriasis. It is also a first-line, or primary, systemic drug used to treat adults with severe psoriasis, von Zumbusch pustular psoriasis, or erythrodermic psoriasis. Neoral is the preparation used most often for psoriasis, and it clears psoriasis in many patients within 8 - 12 weeks.

Side Effects. Cyclosporine has significant side effects if used for a long time, notably kidney problems and non-melanoma skin cancers. It should be reserved for patients who do not respond to phototherapy or less potent systemic medications (for example, methotrexate or acitretin).

Common and temporary side effects include:

- Fatigue
- Gingivitis
- Gout
- Excessive growth of body hair
- Headaches
- Joint pain
- Tremor

More serious complications may include:

- Kidney damage
- High blood pressure (Some doctors advise treating high blood pressure with calcium channel blockers, because other standard blood pressure drugs may worsen psoriasis. Calcium channel blockers also help prevent kidney problems.)
- High cholesterol and lipid levels
- High levels of calcium and low levels of magnesium
- Increased risk for infections
- Liver problems
- Lymphomas (cancers of the lymphatic system)
- Skin cancers (Patients who take cyclosporine after PUVA therapy have a higher incidence of squamous cell skin cancer. The risks are greatest with long-term and previous use of PUVA, methotrexate, or other immunosuppressants.)

Patients should be monitored regularly for high blood pressure and signs of kidney or liver problems and skin cancers.

Patients Who Should not Use Cyclosporine. Because the drug suppresses the immune system, people with active infections or cancer should avoid it. Patients with uncontrolled high blood pressure and impaired kidney function should also not use this medication. Cyclosporine therapy for children with psoriasis has not been well studied.

Drug and Food Interactions. Cyclosporine interacts with numerous drugs -- both prescription and over-the-counter preparations -- as well as grapefruit and grapefruit juice.

Biological Response Modifiers

Biological response modifiers, sometimes called "biologics," belong to a new class of drugs that are considered the most exciting development in psoriasis treatment. Biologics are genetically engineered drugs that interfere with specific components of the autoimmune response. Because of their precise targets, these drugs do not damage the entire immune system like general immunosuppressants. Biologic drugs are expensive.

Biologics are traditionally second- or third-line treatments, and may be used alone or in combination with first-line systemic drugs. Depending on the severity of psoriasis, some of these drugs may be used earlier in the course of treatment. Studies of these medications have primarily been done on patients who are over 18 years old.

There are different types of biologics used to treat psoriasis:

- T-cell blockers block immune cells linked to inflammation.
- Tumor necrosis factor (TNF) blockers target the chemical messenger TNF-alpha, which is released during the inflammatory response.

Types of T-cell blockers:

- Alefacept (Amevive). This drug is approved for the treatment of moderate-to-severe plaque psoriasis. It is very effective for psoriasis of the scalp. However, it doesn't work for all patients. Alefacept is given in a doctor's office or clinic. Patients receive weekly injections for 12 weeks. They need blood tests every 2 weeks to make sure T cell levels do not drop too low. Side effects are generally mild and include sore throat, dizziness, and cough. There have been a few reports of serious infections and cancer.

Types of TNF blockers:

- Etanercept (Enbrel) is approved for the treatment of moderate-to-severe plaque psoriasis, and for people with psoriatic arthritis. The drug is given either alone or in combination with methotrexate. Side effects include infections and lymphoma. Patients inject themselves under the skin once or twice a week for 12 weeks. Continuing etanercept after 12 weeks may lower the severity of disease without increasing infections or side effects. The drug may be effective in psoriasis patients who have not responded to other biologic drugs or other therapies, and it is also effective in patients who have not yet received biologic treatments. Although etanercept has not been studied in children who have psoriasis, it has been shown to be safe and effective for treating children with rheumatoid arthritis.

Side effects and risks of TNF blockers:

- All of the TNF inhibitors carry the potential for an increased risk of serious infections. Upper respiratory infections are the most common infections that occur.
- Uncommon infections caused by fungi and tuberculosis bacteria also boccur in people using anti-TNF medications. In 2009, the FDA issued a warning to healthcare professionals, stressing the need to test for these infections in people using anti-TNF medications who display symptoms of body-wide (systemic) illness. Because these infections are uncommon, previous delays in diagnosis have resulted in death in some patients.
- Patients receiving these drugs are at risk of reactivating old tuberculosis (TB) infections. Patients are also at higher risk for developing TB. The FDA recommends TB screening with a purified protein derivation (PPD) skin test.
- Whether TNF inhibitors increase the risk for lymphoma and skin cancers is a debated issue.

A number of other side effects are also possible.

Monoclonal Antibodies

Human monoclonal antibodies bind to proteins or cells and stimulate the immune system to destroy those cells.

- Ustekinumab (Stelera) was approved by the FDA in 2009 for the treatment of moderate to severe plaque psoriasis. It is given by injection about every 3 months and may be used as first-line treatment. Patients should discontinue use prior to any elective surgery.

Other Second- and Third-Line Treatments

Immunosuppressants. Some immunosuppressants being studied for psoriasis include tacrolimus (Prograf), pimecrolium, and sirolimus. Studies have been limited, however. Side effects of these medications are similar to those of cyclosporine. Pimecrolimus may specifically target the skin and have fewer side effects. (Some immunosuppressants are also being studied as topical treatments.)

Phototherapy

Phototherapy means to treat with light.

When sunlight penetrates the top layers of the skin, the ultraviolet radiation bombards the DNA inside skin cells and injures it. This can cause wrinkles, aging skin, and skin cancers. However, these same damaging effects can destroy the skin cells that form psoriasis patches.

Phototherapy for psoriasis can be given as ultraviolet A (UVA) light in combination with medications, or as variations of ultraviolet B (UVB) light with or without medications. Not everyone is a candidate. For example, phototherapy may not be appropriate for patients who should avoid sunlight or those with very severe psoriasis.

Psoralen and Ultraviolet A Radiation (PUVA)

Ultraviolet A (UVA) is a main part of sunlight. PUVA therapy uses a photosensitizing medication (usually psoralen) in combination with UVA radiation. A photosensitizing medication makes a person more sensitive to light. Treatment with psoralen and UVA is referred to as PUVA. This approach is very powerful and effective in more than 85% of patients who use it. However, it poses a higher risk for skin cancers than treatment with UVB.

Side Effects and Complications of PUVA.

- UVA penetrates the skin more deeply than UVB, so there is a greater danger of deep skin damage, accelerated skin aging, and skin cancers. Anyone who needs to avoid sunlight should not get this treatment.
- The procedure increases the risk for cataracts if the eyes are not protected for up to 24 hours after treatment.

Special Warning on PUVA and Skin Cancers. It has been known for some time that PUVA can change DNA and cause genetic mutations. PUVA is known to increase the risk for squamous cell skin cancer and slightly increase the risk for basal cell skin cancer, both of which are nearly always curable. One study also reported an increased risk of melanoma. The risk for skin cancers is higher in people who have:

- A family or personal history of skin cancer
- Light skin and fair or red hair
- Received radiation or x-ray treatments or taken immune suppressing drugs
- Received more than 200 PUVA treatments

Discussions are under way about discontinuing PUVA treatment of psoriasis. The following are pro and con arguments about the procedure:

- Opponents of PUVA argue that studies suggest a long-term risk for melanoma, starting about 15 years after treatment, particularly in people who receive more than 250 treatments.
- Supporters of PUVA argue that it is not yet known whether the people who developed melanoma experienced sunburn during the procedures, or if they already had risk factors for skin cancers. If so, properly given treatments could still be considered safe for patients without risk factors. They also argue that PUVA is still the most effective treatment for severe psoriasis, and the alternatives are usually very powerful and relatively new drugs that may have even more serious side effects. Furthermore, adding retinoids may protect against skin cancers while increasing the treatment's effectiveness.

- UVB therapy usually requires about 20 - 40 treatments (about three per week). Full results take about 3 weeks.

Side Effects of UVB. The treatment can cause itching and redness. UVB radiation from sunlight is known to increase the risk for skin cancers. There is no strong evidence that UVB treatments pose any risk for skin cancers except on male genitals. This risk, however, can be significant (4.5%) at high doses.

Narrowband Ultraviolet B (NB-UVB) Radiation

Narrowband radiation may be safer than other approaches, and some scientists now believe it should be the first option for patients with chronic plaque psoriasis.

NB-UVB is used without medications and is very strong. Whether it has any effect on the disease process itself is unclear. The light wavelength is between 310 - 312 nanometers, which is the most beneficial part of sunlight.

Exposure times are shorter, but of higher intensity than with broadband UVB. This therapy is probably less likely than PUVA to cause skin cancers.

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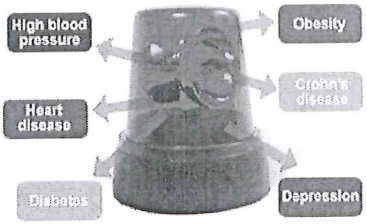
#5

The psoriasis-cancer Link

Having psoriasis may increase the risk of getting certain cancers. But researchers aren't sure why.

By Heather Johnson Durocher

Editor's note: This is the second in a series of ***Psoriasis Advance*** articles about health risks associated with psoriasis. The **first article**, in the Summer 2009 issue, discussed overall health risks. This and future articles will look at individual health conditions (doctors call them co-morbidities) associated with psoriasis.



Psoriasis patients, particularly those dealing with chronically severe forms of the skin disease, are at a higher risk of also having certain types of cancer, according to recent findings.

Both the skin cancer known as squamous cell carcinoma and lymphoma, a cancer that affects the immune system, are health risks some psoriasis patients face, according to a study published last year in the Journal of the American Academy of Dermatology (JAAD).

"Our review found that people with more severe cases of psoriasis appear to have an increased incidence of psoriatic arthritis, cardiovascular disease, hypertension, diabetes, cancer, depression, obesity and even other immune-related conditions such as Crohn's disease," said Dr. Alexa Kimball, one of the study's researchers who serves as vice chair of dermatology at Massachusetts General Hospital and is an associate professor at Harvard Medical School. She's also a member of the National Psoriasis Foundation **Medical Board**.

The risk of certain cancers has troubled the psoriasis community in recent years, said Dr. Ken Gordon, a dermatologist at Evanston Northwestern Healthcare in Skokie, Ill., and also a Psoriasis Foundation Medical Board member.

"The concern we all have is primarily lymphoma, and that's still controversial," Gordon said.

Cause is unclear

Controversial because, while studies like the one published in JAAD have linked a higher risk of lymphoma in patients with severe psoriasis, the actual cause of the cancer remains unclear.

Researchers have hypothesized that the increased rate may be due to the following:

Psoriasis leads to increased T-cell activity because it is a disease that affects the immune system. (T cells are white blood cells in the immune system that fight infections and foreign materials.)

Newer treatments that suppress the immune system (immunosuppressants) may be independent risk factors for developing malignancies.

Or the combination of immunosuppressants and the disease itself may lead to the increased rate.

"We don't know which one is exactly right," said Gordon, who believes long-term studies of psoriasis patients are needed before a definite determination can be made.

"We need better information on the diseases—that's our problem right now. There are some efforts. There is a registry to look at psoriasis patients that is being evaluated. But it's still not as good as registries for rheumatoid arthritis."

Even so, some psoriasis treatments have been linked to a higher risk for cancer. For example, a treatment called PUVA—ultraviolet light A used with a light-sensitive drug called psoralen—is the most clear-cut psoriasis treatment associated with developing cancer, said Dr. Mark Lebwohl, dermatology chairman of the Mount Sinai School of Medicine in New York and the chairman of the National Psoriasis Foundation Medical Board.

There also are some studies showing biologics used to treat psoriasis might be linked to lymphoma and squamous cell carcinoma. It's been known for some time that TNF-alpha blockers are associated with an increase in certain kinds of lymphoma, Lebwohl said. (TNF-alpha blockers suppress a protein in the immune system called tumor necrosis factor-alpha, which is believed to play a role in the development of psoriasis.)

"TNF blockers are the only ones that stop the destruction of the joints (in psoriatic arthritis)," he said. "Other treatments don't do that. When I have patients who will benefit from (a TNF blocker), a patient who has significant psoriatic arthritis for instance, I warn them about the risk of developing lymphomas."

Talking openly with your dermatologist about your psoriasis and your overall health is an important first step in ensuring good health, experts said. Specific to cancer risks, Kimball suggests following the recommended routine health screenings for cancers and avoiding high-risk behaviors that increase the risk of developing some cancers, such as smoking, alcohol abuse and intentional sun exposure.

"That must be a high priority for psoriasis patients who may be at an increased risk for these potentially life-threatening diseases," she said.

Learn more about cancer risks by visiting:

- **American Cancer Society**
- **Leukemia and Lymphoma Society**

Additional information

Psoriasis Drugs Carry New Cancer Warnings

Prescription drugs that block the action of proteins in the immune system called tumor necrosis factor (TNF)—which are believed to play a role in developing psoriasis—must carry updated warning labels with information that TNF blockers may cause leukemia and other cancers in children and adolescents.

The warning will be included on these FDA-approved drugs, commonly used to treat psoriasis and/or psoriatic arthritis: Remicade (generic name infliximab), Enbrel (etanercept), Humira (adalimumab) and Simponi (golimumab).

The FDA imposed the new rule after a safety review indentified 48 cancers in children and adolescents who were prescribed TNF blockers. The FDA acknowledged that it cannot definitively state that these drugs caused the cancers, but also said it could not rule out the possibility.

The agency advises doctors and patients to discuss the risks and benefits of the drugs and to watch for early signs of cancer.

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SECTION B and C ATTACHMENT

#6

WAKE FOREST

Baptist Medical Center

Patients with Severe Psoriasis Suffer Daily, Study Finds

A paper published Sept. 1 in the Journal of the American Academy of Dermatology, Stephen Rapp, Ph.D., Steve Feldman, M.D., Ph.D., and others reported that psoriasis patients had the second-poorest physical functioning and the third-poorest mental functioning of 11 health conditions studied, ranging from hypertension, diabetes and depression to cancer, chronic lung disease and congestive heart failure.

Psoriasis is a chronic disease that leaves the skin red, scaly, uncomfortable and easily irritated. Anyone with psoriasis covering more than 10 percent of his or her body is considered to have a severe case.

Wake Forest®
Baptist Medical Center

Patients with Severe Psoriasis Suffer Daily, Study Finds

Psoriasis significantly reduces quality of life, according to researchers at Wake Forest University Baptist Medical Center.

In a paper published Sept. 1 in the Journal of the American Academy of Dermatology, Stephen Rapp, Ph.D., Steve Feldman, M.D., Ph.D., and others reported that psoriasis patients had the second-poorest physical functioning and the third-poorest mental functioning of 11 health conditions studied, ranging from hypertension, diabetes and depression to cancer, chronic lung disease and congestive heart failure.

The results contradict an earlier study that found the quality of life of psoriasis patients to differ little from the general population.

"Previous studies looked only at patients with mild to moderate psoriasis whose disease was stable," said Rapp, an associate professor of psychiatry and behavioral medicine, and the lead author of the paper. "When we included patients with severe psoriasis in the mix, we found that it is very debilitating, both physically and mentally."

Psoriasis is a chronic disease that leaves the skin red, scaly, uncomfortable and easily irritated. Anyone with psoriasis covering more than 10 percent of his or her body is considered to have a severe case.

The paper noted that psoriasis afflicts about 2 percent of the U.S. population, costs $2 billion to $3 billion a year to treat, and is associated with a higher risk of suicide.

The study looked at 317 psoriasis patients at Wake Forest University Baptist Medical Center who used a standardized form to rate their quality of life in eight areas. The results were compared with quality of life surveys of patients suffering from other diseases, using the same form.

Of 11 other diseases evaluated in the study, the researchers found that only patients with congestive heart failure reported poorer physical functioning than patients with severe psoriasis. Only patients with depression and chronic lung disease reported poorer mental functioning.

"These results indicate that psoriasis can be physically and emotionally debilitating even though it is not life-threatening," Rapp said. "It is not simply a cosmetic nuisance."

Gail M. Zimmerman, president and CEO of the National Psoriasis Foundation, said the paper will help the foundation in its efforts to gain more public attention and support for psoriasis research.

"Psoriasis is a disease that is often misperceived by the public and even the medical community," she said. "This study makes an important contribution in pointing to the debilitating effects of psoriasis."

Although the study population was limited, the authors noted that the distribution of mild, moderate and severe cases within the study group is representative when compared with other surveys of psoriasis patients.

Depending on the case, psoriasis is treated with ointments, light therapy or oral medications. But, Rapp said, the study points out the need for the medical community to develop treatments for psoriasis patients that not only control their disease, but protect their quality of life.

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#7

SECTIONS B,C and E ATTACHMENT

Skin pain and skin discomfort is associated with quality of life in patients with psoriasis.

Journal of the European Academy of Dermatology and Venereology © 2011

The purpose of this study was to investigate the association between skin pain or skin discomfort and HRQoL, and explore whether sleep disturbance and psychological distress were mediators of these associations.

CONCLUSION:

In this study, skin pain and skin discomfort were significantly related to HRQoL when controlling for demographic and clinical characteristics. In addition, sleep disturbance mediated the association between skin pain and HRQoL. An understanding of the complex association among physiological and psychological factors, and HRQoL is clinically important in order to provide proper treatment and care of patients with psoriasis.

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Skin pain and skin discomfort is associated with quality of life in patients with psoriasis.

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Abstract

BACKGROUND:

Patients with psoriasis commonly report severe sensory skin symptoms, sleep disturbance, psychological distress and impaired health related quality of life (HRQoL). However, the complex associations among these factors are poorly investigated in this patient group.

OBJECTIVES:

The purpose of this study was to investigate the association between skin pain or skin discomfort and HRQoL, and explore whether sleep disturbance and psychological distress were mediators of these associations.

METHODS:

A total of 139 psoriasis patients from a university hospital setting participated in this exploratory, cross-sectional study. Data were obtained through interviews and questionnaires (Dermatology Life Quality Index, General Sleep Disturbance Scale, Illness Perception Questionnaire) and analysed using a series of multiple regression analyses. HRQoL was the dependent variable. Independent variables and assumed mediators were entered into the model in a predefined order.

RESULTS:

Skin pain, skin discomfort, sleep disturbance and psychological distress were significantly associated with HRQoL (all $P < 0.05$). Sleep disturbance was a partial mediator for the association between skin pain and HRQoL. No such mediation effect was found in terms of psychological distress. The total model explained 40% of the variance in HRQoL.

CONCLUSION:

In this study, skin pain and skin discomfort were significantly related to HRQoL when controlling for demographic and clinical characteristics. In addition, sleep disturbance mediated the association between skin pain and HRQoL. An understanding of the complex association among physiological and psychological factors, and HRQoL is clinically important in order to provide proper treatment and care of patients with psoriasis.

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Full Text Online WILEY

Skin Pain and Discomfort in Psoriasis: An Exploratory Study of Symptom Prevalence and Characteristics

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Few studies have investigated subjective sensory skin symptoms in patients with psoriasis. The aim of this study was to investigate prevalence and characteristics of psoriasis-related skin pain and discomfort, and evaluate differences in demographic/clinical characteristics among patients with or without skin symptoms. A total of 139 patients was recruited for this exploratory, descriptive, cross-sectional study. Data were obtained through interviews and questionnaires. While 42.6% reported skin pain, 36.7% reported skin discomfort. Mean average symptom intensity score (0–10 numeric rating scale) was 4.4 for pain and 3.5 for discomfort. Unpleasant, surface, sensitive, itchy, and hot/burning were the most common symptom qualities. Sleep was the most severely affected function. No differences were found in demographic characteristics. However, larger proportions of patients with skin symptoms had more severe psoriasis ($p < 0.05$). In conclusion, pain and discomfort are more common and more severe in patients with psoriasis than previously estimated. Key words: pain; discomfort; psoriasis; PASI.

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While clinician-assessed physical signs have been the focus of most psoriasis research, there is increasing interest in the patients' experience of sensory symptoms. The progress and recognition of quality of life (QoL) and symptom management research (1), has led to the acceptance of patients' perception and self-report as the gold standard for studying symptoms. In fact, QoL studies (2–4) have shown that more than 90% of patients with psoriasis report physical skin symptoms.

Patients with psoriasis have been shown frequently to experience skin pain and skin discomfort (5, 6). Pain is a symptom defined as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (7). In a survey of skin disease in general practice 25% of patients with psoriasis reported pain (8). QoL studies (9–13) found that patients with psoriasis reported bodily pain scores comparable to those of patients with heart disease and diabetes (14). In addition, psoriasis patient who were female, older (12), or had less education, chronic co-morbidities (13), more severe psoriasis, and psoriasis of longer duration (9, 10, 12) reported more pain. Of note, none of these studies (8–13, 15, 16) determined whether the pain was specific to psoriasis or other medical conditions. Only two studies (5, 17) found that 26–32% of patients with psoriasis reported that their skin hurt "often or all the time". Patients who were female, older, with less education or had more severe psoriasis, were more likely to report that their skin hurt.

Discomfort is a term commonly used in dermatology research and clinical practice to address sensory skin symptoms. However, no consensus definition of discomfort exists. Previous psoriasis studies applied discomfort either as an umbrella term for skin sensations (i.e. pain; itch; prickling; burning;

tingling; stinging; soreness) and signs (i.e. scaling; suppuration) (13, 18–21), as a symptom synonymous to (20), or distinct from pain (22, 23), or as mental/social distress (18, 24). QoL studies (6, 25, 26) showed that at least 23% of patients reported discomfort from their psoriasis. Of note, these studies did not investigate discomfort in relation to demographic or clinical characteristics.

Symptom characteristics such as intensity, qualities, and interference with function are dimensions of the total pain experience (27). Only a limited number of studies reported pain or discomfort characteristics in patients with psoriasis. Research (21, 28) showed that patients with psoriasis reported pain or discomfort intensity scores in the mild to moderate range. In addition, QoL studies (6, 25, 26) showed that 23% of patients reported at least moderate discomfort and 8% reported extreme discomfort from their psoriasis. Sensitivity, burning/stinging, irritation, and itching in the skin were reported by 39–64% of patients with psoriasis (5, 17). While the studies referred to these sensations as distinct symptoms, pain research considers these sensations as symptom qualities (27). In terms of symptom interference, one study (13) showed that psoriasis-specific physical symptoms (i.e. itch, pain, burning, scaling) were related to poor physical function. Other studies suggested that psoriasis-related discomfort interfered with normal work (6, 25) and sexual life (21).

For a number of reasons, the prevalence and specific characteristics of skin pain and skin discomfort in patients with psoriasis cannot be estimated from existing dermatology research. First, the large studies on skin pain prevalence included hospitalized, older patients with moderate to severe psoriasis. Secondly, several studies (6, 25, 26, 28) did not specify whether pain or discomfort was caused by psoriasis skin lesions or psoriasis arthritis. Thirdly, discomfort characteristics were described for only a rare psoriasis subtype (21). Finally, concepts such as symptoms, qualities, and signs were used inconsistently (5, 17) or collapsed into one overall psoriasis symptom entity (13).

Based on the paucity of research, the purposes of this exploratory study were: (i) to describe the prevalence of skin pain or skin discomfort reported by patients with psoriasis; (ii) to determine whether patients with psoriasis with skin pain, skin discomfort, or no skin pain/discomfort differed in any demographic and clinical characteristics; and (iii) to explore symptom characteristics (i.e. intensity, qualities, interference with function) of psoriasis-related skin pain or skin discomfort.

METHODS

Sample and setting

Patients were recruited prior to a consultation at the inpatient and outpatient dermatology units at a university hospital in Oslo, Norway, between January and September 2006. Patients were included if they: had a psoriasis diagnosis; were ≥ 18 years of age; were able to read and write Norwegian; had Caucasian skin type; and could differentiate skin pain from other bodily pain. Patients were excluded if, at the time of recruitment, they had: clinical signs of infection in psoriasis plaques; concomitant skin diseases that caused pain/discomfort; no clinically visible psoriasis; psychiatric diagnosis or cognitive impairment that prohibited them from completing the self-report questionnaires; or had started hospital treatment (e.g. phototherapy, baths).

One of five research nurses approached the patients in the outpatient or inpatient units and explained the study purpose and procedures. Patients who agreed to participate provided written informed consent. The study was recommended by the Regional Committee for Medical Research Ethics, region south, and approved by the Norwegian Data Inspectorate.

A total of 269 adult patients with psoriasis were registered for consultation in the dermatology units during the study period. Thirty-one patients (11.5%) were not invited to participate in the study due to scheduling conflicts. Of the 238 patients approached, 4 did not meet the inclusion criteria for the following reasons: uncertain psoriasis diagnosis (n = 1), non-Caucasian skin type (n = 2), and Norwegian illiteracy (n = 1). In addition, 42 patients were excluded due to: concomitant skin diseases (n = 16), ongoing hospital treatment (n = 14), no psoriasis plaques at the time of recruitment (n = 5), psychological problems (n = 4), and cognitive impairment (n = 3). Of a total of 192 eligible patients, 140 (72.9%) agreed to participate. After enrolment, one patient was excluded due to a change in diagnosis. The final sample therefore included 139 patients.

Study procedures

For each patient, a 30–40 min interview was conducted by one investigator (TML). Patients were screened for skin pain and skin discomfort. Specific definitions of pain and discomfort were not provided, because this exploratory study aimed to investigate the patients' subjective experience of sensory skin symptoms without biasing their responses. During the interview, information was collected on demographic and clinical characteristics. A clinical evaluation of psoriasis severity was carried out and medical records were reviewed for disease and treatment information. Patients were shown how to complete the questionnaires (within 24 h) and return them in postage paid envelopes. The postal questionnaire response rate was 90.6%.

Instruments

Demographic characteristics. Information was collected on gender, age, ethnicity, marital status, living arrangements, education, and employment status.

Co-morbidity. Patients completed the Self-Administered Comorbidity Questionnaire (SCQ-18), which evaluated the number, severity, and functional impact of health problems (29). In this study, the SCQ contained 16 defined and 2 optional conditions (i.e. hypertension, diabetes, abdominal ulcer, headache, depression, osteoarthritis, rheumatoid arthritis, back/neck pain, cancer, musculoskeletal condition, and disease of the heart, lung, bowel, kidney, liver, blood). The total score range from 0 to 54. Higher scores indicate a more severe co-morbidity profile.

Prevalence of skin pain and skin discomfort. Two questions were used to screen patients into three skin symptom groups (i.e. pain, discomfort, no pain/discomfort). First, patients indicated, using a yes/no format, whether they had experienced any skin pain or skin discomfort during the past 24 h. Patients who responded yes indicated whether the sensation was pain or discomfort. Patients who reported both pain and discomfort (n = 3) were categorized in the pain group.

Intensity of skin pain and skin discomfort. The Norwegian version of the Brief Pain Inventory (BPI) (30) was used to measure intensity of pain or discomfort on 0 (no symptom) to 10 (worst symptom imaginable) numeric rating scales (NRS). Four items address present, worst, least, and average pain or discomfort intensity over the past 24 h (30, 31). Suggested NRS cut-points for mild, moderate, and severe chronic non-malignant pain are 1–3, 4–6, and 7–10, respectively (32–35).

Qualities of skin pain and skin discomfort. The Pain Qualities Assessment Scale (PQAS) (36) was used to assess pain or discomfort qualities. For the purpose of this study, PQAS was translated from English to Norwegian using the Linguistic Validation method (37). This questionnaire includes 20 items that evaluate symptom intensity, quality, spatial characteristics, and temporal pattern over the past week. Each quality's severity is scored 0 (no/not [item]) to 10 (the most [item] sensation imaginable) on a NRS.

These continuous variables were dichotomized into "not endorsed" (0) or "endorsed" (> 0) in order to determine the percentage of patients who endorsed particular symptom qualities.

Interference of skin pain and skin discomfort. The BPI (30) was used to evaluate interference of skin symptoms on a 0 (no interference) to 10 (worst interference imaginable) NRS. Seven items address symptom interference with daily activities, mood, walking ability, work, relations with other people, sleep, and enjoyment of life over the past 24 h (30, 31). Interference scores ≥ 4 indicate clinically significant interference with function in cancer patients (38, 39). The continuous variables of interference severity were dichotomized into "not endorsed" (0) or "endorsed" (> 0) in order to determine the percentage of patients who endorsed particular symptom interference items.

Clinical evaluation

Duration and severity of psoriasis. Psoriasis duration in years was obtained through patient interviews. Psoriasis severity was determined using the Psoriasis Area and Severity Index (PASI). The PASI total score ranges from 0–72. Higher scores indicate greater psoriasis severity (40).

Overall psoriasis condition. Patients rated their overall condition of psoriasis as stable, improved, or exacerbated (41).

Medications and topical treatments for psoriasis. Information was collected on medication (i.e. non-prescription, prescription) and psoriasis treatment through patient interviews and from medical records. Treatments were collapsed into two groups (i.e. topical and/or phototherapy, or systemic alone or with topical- and/or phototherapy).

Statistical analysis

Data were analysed using the SPSS for Windows version 16.0 (SPSS, Inc., Chicago, US) and SPSS SamplePower 2.0. Descriptive statistics were generated on demographic, clinical, and symptom characteristics. Differences among the pain (n = 58), discomfort (n = 51), and no pain/discomfort (n = 30) groups in categorical variables were examined with $\chi 2$ and Fisher's exact test analyses. Differences among the three groups in continuous variables were examined with one-way analysis of variance (ANOVA) and Kruskal Wallis analyses. Nine pairwise contrasts were calculated to locate group differences in the categorical variable "overall psoriasis condition". The significance criterion for each of these contrasts was set at 0.006 (0.05/9). Three pairwise contrasts were calculated to locate group differences in the mean PASI score. The significance for each of these contrasts was set at 0.017 (0.05/3). For all other variables, a p-value of < 0.05 was considered statistically significant. 95% confidence intervals (95% CI) were generated for the proportions of patients who reported skin pain or skin discomfort.

RESULTS

Prevalence

In this sample, 41.7% (95% CI, 33.8–50.0%) of patients reported skin pain and 36.7% (95% CI, 29.1–45.0%) of the patients reported skin discomfort.

Differences in demographic and clinical characteristics

The majority of the patients were women (56.8%), married (61.2%), and working (60.4%). Their mean age was 51.4 years (± 13.2; range 18–84 years). No differences were found among the three groups on any of the demographic characteristics (Table I).

Table I. Differences in demographic characteristics among patients with pain, discomfort and no pain/discomfort

Characteristics	Pain	Discomfort	No pain/ discomfort	Statistics
	n = 58 (41.7%)	n = 51 (36.7%)	n = 30 (21.6%)	
Age (years), mean ± SD	49.7 ± 13.8	51.8 ± 13.6	54.1 ± 11.3	F = 1.136 (p = 0.324)
Gender, % (n)				
Male	39.7 (23)	37.3 (19)	60.0 (18)	$\chi2$ = 4.483 (p = 0.106)
Marital status, % (n)				
Unmarried	17.2 (10)	15.7 (8)	10.0 (3)	
Married/cohabitant	53.4 (31)	66.7 (34)	66.7 (20)	p = 0.531a
Divorced/widowed	29.3 (17)	17.6 (9)	23.3 (7)	
Living arrangement, % (n)				
Alone	27.6 (16)	25.5 (13)	16.7 (5)	
Spouse	32.8 (19)	39.2 (20)	60.0 (18)	$\chi2$ = 6.179 (p = 0.186)
Family/others	39.7 (23)	35.3 (18)	23.3 (7)	
Employment status, % (n)				
Employed	56.9 (33)	60.8 (31)	66.7 (20)	$\chi2$ = 0.794 (p = 0.672)
Unemployed	43.1 (25)	39.2 (20)	33.3 (10)	
Education, % (n)				
Primary school	12.1 (7)	15.7 (8)	23.3 (7)	
Secondary school	44.8 (26)	47.1 (24)	23.3 (7)	p = 0.175a
University ≤ 4 years	20.7 (12)	27.5 (14)	33.3 (10)	
University > 4 years	22.4 (13)	9.8 (5)	20.0 (6)	
Ethnicity, % (n)	91.4 (53)	98.0 (50)	93.3 (28)	p = 0.329a

Norwegian	8.6 (5)	2.0 (1)	6.7 (2)
Other			

aFisher's exact test. SD: standard deviation.

The mean SCQ-18 score was 5.4 (± 4.3). Back/neck pain (38.8%), headache (28.8%), hypertension (24.5%), rheumatoid arthritis (22.3%), and depression (17.3%) were the most common co-morbidities. No differences were found among the three groups in terms of co-morbidity profile.

The mean PASI score was significantly higher in patients with pain (7.1 ± 5.8) and discomfort (5.4 ± 4.1) than patients with no pain/discomfort (2.7 ± 2.4) (all $p < 0.01$) (Table II). Higher percentages of patients in the pain and discomfort groups reported exacerbated psoriasis condition compared with those in the no pain/discomfort group ($p < 0.006$). Furthermore, a higher percentage of patients in the no pain/discomfort group reported improved psoriasis condition compared with those in the pain and discomfort groups ($p < 0.006$). No differences were found among the three groups in terms of mean duration of psoriasis, medication and topical treatment.

Table II. Differences in endorsed qualitites and interference items between patients with pain, discomfort and no pain/discomfort

Characteristics	Pain (1) n = 58 (41.7%)	Discomfort (2) n = 51 (36.7%)	No pain/discomfort (3) n = 30 (21.6%)	Statistics
SCQ-18 (0–54), mean ± SD	5.6 ± 4.5	5.3 ± 4.1	5.2 ± 4.6	KWχ2 = 0.385 ($p = 0.825$)
Co-morbidity score				
PASI (0–72), mean ± SD	7.1 ± 5.8	5.4 ± 4.1	2.7 ± 2.4	KWχ2 = 20.648 ($p < 0.0001$) 1 and 2 > 3a
Ps overall condition, % (n)	22.4 (13)	25.5 (13)	43.3 (13)	χ2 = 29.772 ($p < 0.0001$)
Stable	13.8 (8)	17.6 (9)	50.0 (15)	Not significantb
Improved	63.8 (37)	56.9 (29)	6.7 (2)	1 and 2 < 3b
Exacerbated				1 and 2 > 3b

aStatistically significant pairwise contrast $p < 0.017$.

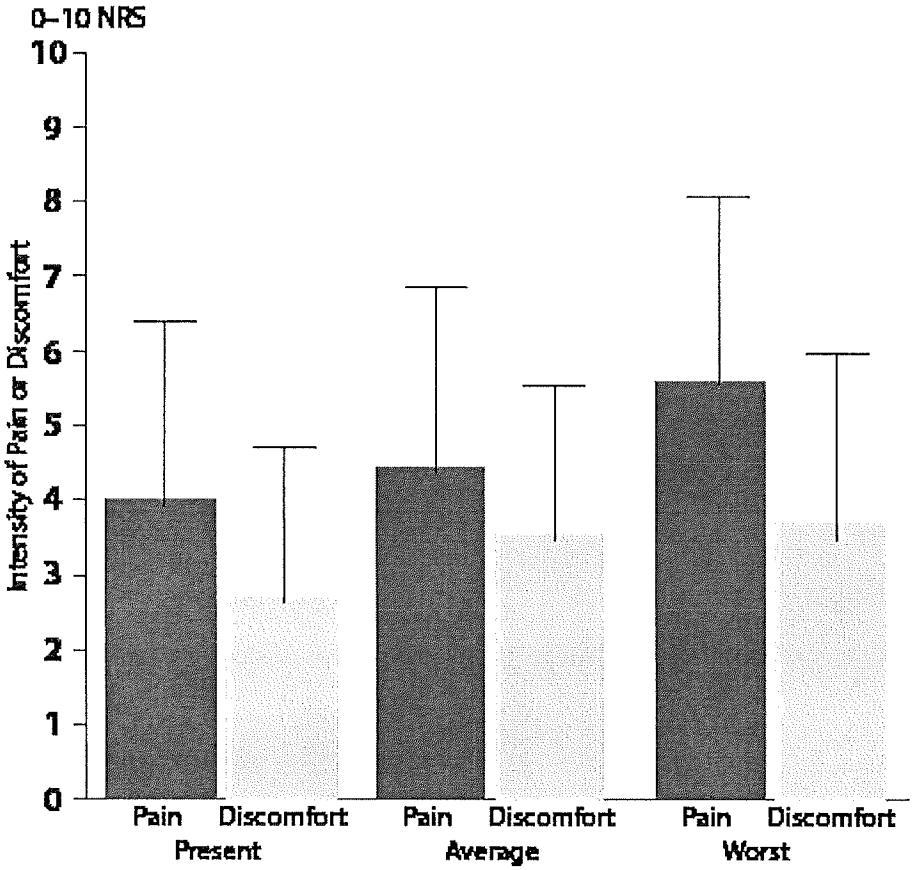
bStatistically significant pairwise contrast $p < 0.006$.

KW: Kruskal Wallis test; MU: Mann-Whitney U test; PASI: Psoriasis Area and Severity Index; Ps: psoriasis; SCQ: Self-Administered Comorbidity Questionnaire; SD: standard deviation.

Symptom characteristics

Mean symptom intensity scores (0–10 NRS) ranged from 4.0 to 5.6 in the pain group, and from 2.7 to 3.7 in the discomfort group (Fig. 1).

Fig. 1. Present, worst, and average intensity (mean and standard deviation) of skin pain and skin discomfort over the past 24 h in patients with psoriasis. NRS: numeric rating scale.



Except for the quality "throbbing", no significant differences were found between the patients with pain or discomfort in any of the symptom qualities they endorsed (Table III). In both groups, the most frequently reported qualities were unpleasant (100%), surface (99%), sensitive (96%), itchy (96%), hot/burning (93%), tender (84%), and tingling (79%). The mean severity scores (0–10 NRS) for these seven most common symptom qualities were significantly higher for the pain than the discomfort group (all $p < 0.05$). The severity scores ranged from 4.4 to 6.6 in patients with pain, and from 2.7 to 5.2 in patients with discomfort (Table IV).

Table III. Differences in endorsed qualities between patients with pain and discomfort

Symptom characteristics	Pain	Discomfort	Statisticsb

	n = 58	n = 51	
Qualitiesa, % (n)			
Itchy	95.8 (46)	95.8 (46)	p = 1.000
Unpleasant	100.0 (49)	100.0 (48)	p = 1.000
Surface	98.0 (48)	100.0 (48)	p = 0.362
Sensitive	97.9 (47)	93.6 (44)	p = 0.268
Hot/burning	95.9 (47)	89.6 (43)	p = 0.089
Tender	91.5 (43)	76.6 (36)	p = 0.077
Tingling	87.5 (42)	70.8 (34)	p = 0.162
Aching	81.3 (39)	68.1 (32)	p = 0.352
Sharp	79.2 (38)	70.2 (33)	p = 0.301
Cramping/tight	63.8 (30)	52.1 (25)	p = 0.010
Throbbing	77.6 (38)	51.1 (24)	p = 1.000
Deep	70.2 (33)	68.8 (33)	p = 0.100
Heavy	64.6 (31)	45.8 (22)	p = 0.664
Radiating	63.0 (29)	68.8 (33)	p = 0.153
Shooting	57.4 (27)	41.7 (20)	p = 0.678
Dull	62.5 (30)	57.4 (27)	p = 0.297
Cold	44.7 (21)	33.3 (16)	p = 0.838
Numb	51.1 (24)	47.9 (23)	p = 1.000
Electric	34.0 (16)	35.4 (17)	
Interference with functiona, % (n)	85.7 (42)	66.7 (32)	p = 0.033
Sleep	91.8 (45)	79.2 (38)	p = 0.090
Enjoyment of life	93.9 (46)	77.1 (37)	p = 0.022
Mood	91.7 (44)	66.7 (32)	p = 0.005
Work	85.7 (42)	72.9 (35)	p = 0.139
Daily activities	87.8 (43)	54.2 (26)	p < 0.001

Relationships with other people	77.6 (38)	62.5 (30)	p = 0.124
Walking ability			

a"Endorsed" > 0 on an 11-point numeric rating scale (NRS).

bFisher's exact test.

Significant values are shown in bold.

Table IV. Differences in severity of qualities between patients with pain and discomfort

Symptom characteristics	Pain n = 58 (53.2%)	Discomfort n = 51 (46.8%)	Statistics
Severity of qualities (0–10 NRS), mean ± SD			
Itchy	6.6 ± 3.2	5.2 ± 2.5	t = 2.4, p = 0.019
Unpleasant	6.0 ± 2.3	4.4 ± 2.3	t = 3.3, p = 0.001
Surface	6.0 ± 2.5	4.2 ± 2.3	t = 3.8, p < 0.001
Sensitive	5.9 ± 2.6	4.3 ± 2.7	t = 2.9, p = 0.005
Hot/burning	5.6 ± 2.8	3.7 ± 2.5	t = 3.6, p < 0.001
Tender	4.9 ± 2.7	3.2 ± 3.0	t = 2.9, p = 0.005
Tingling	4.4 ± 3.0	2.7 ± 2.7	t = 3.1, p = 0.003
Aching	3.9 ± 3.1	2.2 ± 2.3	t = 3.1, p = 0.003
Sharp	3.6 ± 2.9	2.5 ± 2.6	t = 1.9, p = 0.062
Cramping/tight	3.6 ± 3.5	1.9 ± 2.6	t = 2.7, p = 0.008
Throbbing	3.2 ± 2.9	1.6 ± 2.2	t = 3.0, p = 0.003
Deep	3.2 ± 2.9	2.1 ± 2.2	t = 1.9, p = 0.056
Heavy	3.2 ± 3.2	1.6 ± 2.2	t = 2.8, p = 0.006
Radiating	2.7 ± 3.0	2.0 ± 1.9	t = 1.4, p = 0.160
Shooting	2.5 ± 3.1	1.3 ± 2.1	t = 2.1, p = 0.037
Dull	2.2 ± 2.4	1.9 ± 2.2	t = 0.6, p = 0.558
Cold	2.0 ± 2.8	1.0 ± 1.9	t = 1.9, p = 0.058

Numb	1.8 ± 2.5 1.8 ± 2.6	t = 0.0, p = 0.975
Electrical	1.4 ± 2.5 1.1 ± 2.0	t = 0.6, p = 0.582
Severity of interference (0–10 NRS), mean ± SD		
Sleep	5.0 ± 3.1 2.6 ± 2.6	t = 4.1, p < 0.001
Enjoyment of life	4.9 ± 3.2 2.7 ± 2.6	t = 3.9, p < 0.001
Mood	4.8 ± 2.7 2.5 ± 2.5	t = 4.4, p < 0.001
Work	4.8 ± 3.0 2.7 ± 2.7	t = 3.5, p = 0.001
Daily activities	4.5 ± 2.8 2.7 ± 2.4	t = 3.3, p = 0.001
Relationships with other people	3.9 ± 2.9 1.9 ± 2.3	t = 3.8, p < 0.001
Walking ability	3.8 ± 3.0 2.7 ± 3.2	t = 1.8, p = 0.072

Significant values are shown in bold.

NRS: numeric rating scale; SD: standard deviation.

Significantly higher percentages of patients with pain (88–94%) reported that their symptom interfered with mood, work, sleep, and relations with other people compared with patients with discomfort (54–77%) (all $p < 0.05$) (Table III). Mean severity scores (0–10 NRS) for the interference items ranged from 3.8 to 5.0 in patients with pain and from 1.9 to 2.7 in patients with discomfort (Table IV). Patients with pain reported significantly higher severity scores on all interference items (all $p \leq 0.001$), except from walking ability.

DISCUSSION

In this study, almost 80% of patients with psoriasis reported sensory skin symptoms. Patients with skin pain or skin discomfort had more severe psoriasis than those with no skin pain/discomfort. Patients with skin pain reported mean pain intensity scores in the moderate range (32–35). Patients used common qualities such as unpleasant, surface, and sensitive to describe both pain and discomfort. In addition, sleep and enjoyment of life were the functions most severely affected by sensory skin symptoms.

The CI for symptom prevalence in the present study indicate that the population prevalence of skin pain probably ranges from 34% to 50% in patients who attend hospital care for psoriasis. Furthermore, the population prevalence of skin discomfort probably ranges from 29% to 45% in this patient population. The width (± 8%) of these CI is relatively narrow. In fact, even the lower limits of these CI indicate higher symptom prevalence compared with estimates from previous research (5, 17, 25). This difference may be attributed to the fact that patients in the present study were asked directly about skin pain and discomfort rather than having the symptom included in a list of skin symptoms (42). In addition, the lower rates in other studies may be due to the exclusion of patients who reported milder symptom severity (25) or symptoms less frequent than "often" (5, 17).

In the present study, larger proportions of patients with pain or discomfort had more severe psoriasis, as well as exacerbation of skin disease, compared with patients with no pain/discomfort. These findings

are supported by previous research (9, 10, 12). Of note, both skin lesions (e.g. a defective skin barrier, inflammation) and treatments are potential sources of pain and discomfort. However, in the present study patients did not specify whether the psoriasis itself or the treatments for psoriasis caused skin pain or discomfort.

In the present study, patients rated mean skin pain intensity scores in the moderate range (32–35) (Fig. 1). The ratings of average and worst skin pain were equivalent to pain intensity scores reported by patients with neuropathic pain conditions (32, 43). Cut-points are not established for discomfort intensity measured on 0–10 NRS. However, in the present study patients rated discomfort intensity at the lower end of the NRS (Fig. 1), which may be interpreted as mild symptom intensity. Of note, while all patients who reported discomfort were included in this study's analyses, previous studies (6, 25, 26) included only patients who reported at least moderate discomfort. Methodological differences make it difficult to compare findings on discomfort intensity across studies.

Patients with skin pain and skin discomfort endorsed common symptom qualities (Table III). However, patients with pain reported higher severity scores for a majority of these qualities (Table IV). The most frequent and severe qualities reported by both groups may provide insight into a "set" of specific skin symptom qualities in patients with psoriasis. The "set" includes the affective quality of "unpleasant" and the spatial quality of "surface" rather than "deep". In addition, the sensory qualities of "hot/burning", "sensitive", and "tender" are, from a clinical angle, consistent with psoriasis characteristics such as inflammation and skin trauma. "Sensitive", "hot/burning", and "itchy" are also common and severe qualities reported by patients with neuropathic pain conditions (44, 45). Of note, the type of skin pain (e.g. nociceptive, inflammatory, neuropathic) (46) in patients with psoriasis cannot be determined from this study. Further research that utilizes quantitative sensory testing and skin biopsies may provide such insight.

Larger proportions of patients with pain reported that their skin symptom interfered with function (Table III). These patients also reported more severe interference with function compared with patients with discomfort (Table IV). In fact, 5 of the 7 mean interference severity scores in the pain group were above 4, which suggests a clinically significant level of interference (38, 39). These findings are comparable to pain interference ratings reported by outpatients with cancer (47, 48) diabetic neuropathy (49), and herpes zoster (50). Interestingly, "sleep" and "enjoyment of life" were the interference items with the highest severity scores in the total sample. Previous research (51, 52) suggests that sleep impairment may be a major problem for patients with psoriasis. Future studies need to examine in depth the association between skin symptoms and sleep disturbance. While previous QoL studies (13) show impaired function in patients with psoriasis, sensory skin symptoms' impact on function was not investigated. Of note, recent research (53) questions patients' ability to distinguish the cause of impaired function. Further studies are needed in order to establish the extent that skin sensory symptoms vs. other disease features have on interference with function.

Patients with pain, discomfort, or no pain/discomfort did not differ on any demographic characteristics. However, previous studies suggest that female gender is associated with pain in patients with psoriasis (2, 5, 12, 17). Recent research (54) showed that pain thresholds and pain tolerance levels were lower in women. Biological as well as psychological differences between the genders were suggested explanations for the higher prevalence and severity of pain found in women. The sample size of the present study may have been too small to detect gender differences.

Patients with or without skin symptoms did not differ in terms of age and co-morbidity profile, as opposed to findings from previous research (2, 5, 12). A possible explanation for the present study's

findings may be that skin pain and discomfort from psoriasis was assessed specifically rather than pain and discomfort in general (10–15, 17–19, 28, 46). Therefore, the patients' report of psoriasis related skin pain and discomfort did not account for coexisting pain and discomfort from co-morbidities associated with older age.

Some limitations of the present study are worth noting. The relatively small sample size may have precluded us from finding associations between demographic or clinical characteristics and pain and discomfort (2, 5, 17). Although patients were from several regions of Norway and had mild to severe psoriasis, findings may not be generalizable to general and private practice since the sample was recruited from one hospital dermatology unit. Finally, because of the methods used for quantifying symptom characteristics, a more comprehensive evaluation of the relation between pain and discomfort could not be performed.

In conclusion, findings from this study suggest that psoriasis-related skin pain and skin discomfort may be a larger problem than previously estimated.

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The authors declare no conflict of interest.

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#8

SECTION B,C and E attachment

As chronicled on ABC News 20 June 2011

People who suffer from itching that lasts weeks, months or even years, the discomfort can be debilitating. According to a new study, in fact, it can be just as debilitating as chronic pain.

Dr. Suephy Chen, associate professor of dermatology at the Emory University School of Medicine and a physician at the Atlanta VA Medical Center. Chen is also a co-author of the study published in the current issue of the journal Archives of Dermatology. chronic itch to subjects with chronic pain and discovered that both conditions are equally as debilitating. Certain medical conditions, such as eczema and psoriasis, can cause itching. Chronic itching can also be idiopathic, meaning there's no known cause of it. Regardless of the reason behind it, people with the condition often experience depression, anxiety and difficult sleeping.

Itching, unlike other skin sensations, is generally a result of CNS activities and typically goes untreated by standard medical therapies.

ABC NEWS

Chronic Itching and Chronic Pain Equally as Debilitating

Both Conditions Interfere With People's Lives, Says Study

By KIM CAROLLO, ABC News Medical Unit

June 20, 2011—

Anyone who has had a mosquito bite knows how maddening the relentless itching can be, even if it only lasts a few days.

But for people who suffer from itching that lasts weeks, months or even years, the discomfort can be debilitating. According to a new study, in fact, it can be just as debilitating as chronic pain.

Leigh, who didn't want her last name used, recently suffered from a bout of persistent itching that lasted for about two months. At first, doctors couldn't find the cause, so she was stuck trying various creams that didn't work.

"It distracted me from everything I did," she said. "I was constantly scratching or thinking about scratching."

Eventually, after a third trip to the doctor, she got a diagnosis: scabies, a condition that causes severe itching as a result of mites burrowing into the skin.

The condition went away a couple of months later, but only after she shelled out a lot of money for ineffective creams and scratched through many sleepless nights.

"Itching isn't much different than pain. Both impact quality of life," said Dr. Suephy Chen, associate professor of dermatology at the Emory University School of Medicine and a physician at the Atlanta VA Medical Center. Chen is also a co-author of the study published in the current issue of the journal Archives of Dermatology.

Chen and her fellow researchers wanted to find out how much chronic itching impacts people's lives. They compared subjects with chronic itch to subjects with chronic pain and discovered that both conditions are equally as debilitating. They defined chronic itching as anything lasting longer than six weeks.

Certain medical conditions, such as eczema and psoriasis, can cause itching. Chronic itching can also be idiopathic, meaning there's no known cause of it. Regardless of the reason behind it, people with the condition often experience depression, anxiety and difficult sleeping.

Study participants found their itching such a detriment to their quality of life that they indicated they were willing to give up 13 percent of their life span -- about 10 years, based on how long the average American lives -- to live itch-free.

The study also found that being married helped people deal with their situation better.

"Being married helped because they have a support system at home," said Chen. For people who aren't married, "having some other support system can be helpful."

Chronic Itching 'Underappreciated'

Support is vital because coping with chronic itching can be very difficult. Chen said she has several patients who have gotten divorced because their partners couldn't understand why the scratching wouldn't stop.

"The impact of itching is underappreciated," said Dr. Robert Kirsner, professor and vice president of the Department of Dermatology at the University of Miami's Miller School of Medicine. "It can have severe effects on quality of life and this work serves to highlight its importance."

"If you think about medical conditions that people pay attention to, like cancer, people can relate to that," Kirsner added. "It's hard to relate to someone itching."

Chronic itching is also difficult to treat, unlike itching that lasts only a short time as well as chronic pain.

"There are a lot of options for pain control," said Dr. Jennifer Stein, assistant professor of dermatology at NYU Langone Medical Center in New York. "But for people who have chronic itching, there are fewer options.

"It's especially bad at night," Stein added. "Sometimes, during the day you can preoccupy yourself with daily activities, but at night, there's not much to distract you from the itching."

Constant scratching can cause rashes, redness or cuts, and infection can set in. It can also be stigmatizing.

"It's fairly socially unacceptable to sit there scratching," said Chen.

Chen and other experts hope this study can lead to the development of more effective treatments for chronic itching.

That would be great news for Leigh, although she has one much bigger hope.

"I hope to never go through anything like that again," she said. "It was absolutely horrible."

#9

SECTION C ATTACHMENT

According to JAAD *Journal of the American Academy of Dermatology*

The impact of psoriasis on HRQL is similar to that of other major medical diseases. Different aspects of psoriasis are related to the different dimensions of HRQL supporting the need for multidimensional treatment models. (J Am Acad Dermatol 1999;41:401-7.)

« Previous

Journal of the American Academy of Dermatology
Volume 41, Issue 3 , Pages 401-407, September 1999

Next »

Psoriasis causes as much disability as other major medical diseases☆☆☆★★★

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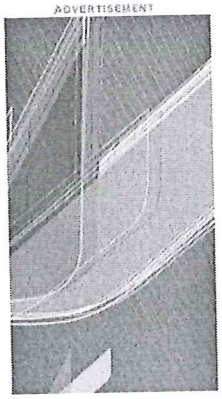
Accepted 1 April 1999

Abstract

Background: Little is known about how the health-related quality of life (HRQL) associated with psoriasis compares with that of other patient populations. **Objective:** We describe HRQL associated with psoriasis and compare it with HRQL of patients with other major chronic health conditions. A second aim is to identify which specific aspects of psoriasis contribute most to HRQL. **Methods:** A total of 317 patients completed a non-disease-specific measure of HRQL. Responses were compared with those of patients with 10 other chronic health conditions. HRQL was regressed on ratings of 18 aspects of psoriasis. **Results:** Patients with psoriasis reported reduction in physical functioning and mental functioning comparable to that seen in cancer, arthritis, hypertension, heart disease, diabetes, and depression. Six aspects of psoriasis predicted physical functioning, and 5 different disease aspects predicted mental functioning. **Conclusion:** The impact of psoriasis on HRQL is similar to that of other major medical diseases. Different aspects of psoriasis are related to the different dimensions of HRQL supporting the need for multidimensional treatment models. (J Am Acad Dermatol 1999;41:401-7.)



10

ATTACHMENT FOR SECTIONS G and H

This is a companion article from ***MEDICAL NEWS TODAY*** being referenced here to the article written and presented here titled ***Skin Pain and Skin Discomfort is Associated With Quality of Life in Patients With Psoriasis*** from the National Center for Biotechnology at The U.S. Library of Medicine. ***The Skin Pain and Skin Discomfort is Associated With a Quality of Life in Patients With psoriasis*** article clearly points out that sleep disturbance, psychological distress and pain are problem issues for many suffering from psoriasis, a chronic and debilitating disease. The below Article from *Medical News Today* is titled ***Smoking Cannabis Reduces Pain, Helps Sleep And Improves Mood For Those With Chronic Symptoms***

ARTICLE

Smoking Cannabis Reduces Pain, Helps Sleep And Improves Mood For Those With Chronic Symptoms

Last updated on Tuesday 18 June 2013

Originally published on Monday 30 August 2010

Editors' Choice

For patients with chronic (long-term) neuropathic pain, smoking cannabis was found to reduce symptoms of pain, improve mood and help sleep, a report published in *CMAJ* (Canadian Medical Journal Association) revealed. When damage or dysfunction of the nervous system results in chronic neuropathic pain, patients have few treatment options, such as antidepressants, local anesthetics, anticonvulsants or opioids. However, these medications often have undesirable side effects and do not work for everybody.

However, researchers from the Mayo Clinic said medical marijuana is not recommended for teens with chronic pain.

The authors in the article published in *CMAJ* inform that oral cannabinoids have been effective in reducing the symptoms of some types of pain. However, they many have different effects and risks compared to smoked cannabis.

Investigators from McGill University Health Centre (MUHC) and McGill University carried out a randomized, controlled trial to determine the analgesic effect of smoked cannabis in 21 patients, aged 18 years or more, all of them with chronic neuropathic pain. THC levels (drug potencies) were divided into 2.5%, 6% and 9.4%. Some participants also received a placebo (0%).

The researchers inform that there was a correlation between increased THC content and better sleep quality. Symptoms of depression and/or anxiety were also reduced at 9.5% THC level.

Lead author Dr. Mark Ware, Director of Clinical Research at the Alan Edwards Pain Management Unit of the MUHC, said:

We found that 25 mg herbal cannabis with 9.4% THC, administered as a single smoked inhalation three times daily for five days, significantly reduces average pain intensity compared with a 0% THC cannabis placebo in adult subjects with chronic post traumatic/post surgical neuropathic pain. We found statistically significant improvements in measures of sleep quality and anxiety.

The authors believe their study is the *"first outpatient clinical trial of smoked cannabis ever reported."* As there have not been many studies on smoked cannabis for neuropathic pain, the investigators say there should be further, longer-lasting trials with higher THC potencies. Long-term safety studies of smoked cannabis for medical purposes are also needed, they added.

Dr. Henry McQuay of Balliol College, Oxford University, UK, writes in a related Commentary:

The authors should be congratulated for tackling such a worthwhile question as: does cannabis relieve neuropathic pain?, particularly because the trial must have been a major nightmare to get through the various regulatory hurdles. What makes it a worthwhile question is the continuing publicity that patients see, hear and read, suggesting analgesic activity of cannabis in neuropathic pain, and the paucity of robust evidence." He concludes that "this trial adds to the trickle of evidence that cannabis may help some of the patients who are struggling at present.

"Smoked cannabis for chronic neuropathic pain: a randomized controlled trial"
Mark A. Ware, Tongtong Wang, Stan Shapiro, Ann Robinson, Thierry Ducruet, Thao Huynh, Ann Gamsa, Gary J. Bennett, Jean-Paul Collet
Published online ahead of print August 30, 2010
CMAJ 10.1503/cmaj.091414

Commentary: ***"More evidence cannabis can help in neuropathic pain"***
Henry J. McQuay
Published online ahead of print August 30, 2010
CMAJ 10.1503/cmaj.100799

Written by Christian Nordqvist

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The purpose of this document and all attached and or accompanying supporting documents is for the purpose of including Psoriasis and Psoriatic Arthritis to the List of Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.

Section G: Attachment – General Evidence of Support for Medical Marijuana

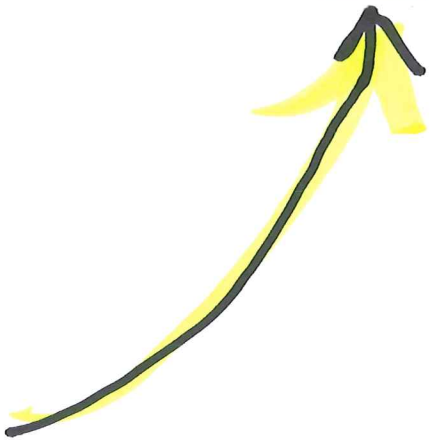
Attached is a direct listing showing Psoriasis is a recognized and approved condition treatable under the Arizona Medical Marijuana program

Arizona Medical Marijuana Blog Medical Conditions Treated With Marijuana

Posted on July 23, 2012 by Kevin

Medical Conditions Treated With Marijuana Medical Conditions for Marijuana Treatment Acquired Hypothyroidism Agoraphobia – (from Greek ἀγορά, "marketplace"; and φόβος/φοβία, -phobia) is an anxiety disorder characterized by anxiety in situations where the sufferer perceives the environment to be difficult or ... Continue reading →

Posted in Acquired Hypothyroidism, Adenocarcinoma, Adrenal Cortical Cancer, Agoraphobia, AIDS, Alcohol Abuse, Alcoholism, Alopecia Areata, Alzheimer's Disease, Amphetamine Dependency, Amyloidosis, Amyotrophic Lateral Sclerosis ALS, Angina Pectoris, Ankylosis, Anorexia Nervosa, Anxiety Disorders, Arteriosclerotic Heart Disease, Arthritis, Arthropathy - Gout, Asthma, Attention Deficit Hyperactivity Disorder ADD/ADHD, Autism - Aspergers, Autoimmune Disease, Bell's Palsy, Bipolar Disorder, Brain Tumor - Malignant, Bruxism, Bulimia Nervosa, Cachexia, Cancer Treatments, Cannabis and Insomnia, Carpal Tunnel Syndrome, Cerebral Palsy, Cervical Disk Disease, Cervicobrachial Syndrome, Chemotherapy, Chronic Fatigue Syndrome, Chronic Pain, Chronic Renal Failure, Cocaine Dependence, Colitis, Conjunctivitis, Constipation, Crohn's Disease, Cystic Fibrosis, Darier's Disease, Degenerative Arthritis, Degenerative Arthropathy, Delirium Tremens, Depression, Dermatomyositis, Diabetes - Adult Onset, Diabetes - Insulin Dependent, Diabetic Neuropathy, Diabetic Peripheral Vascular Disease, Diarrhea, Diverticulitis, Dreaming, Dysthymic Disorder, Eczema, Emphysema, Endometrial Cancer, Endometriosis, Epidermolysis Bullosa, Epididymitis, Epilepsy, Felty's Syndrome, Fibromyalgia, Friedreich's Ataxia, Gastritis, Genital Herpes, Glaucoma, Glioblastoma Multiforme, Graves Disease, Hemophilia A, Henoch-Schonlein Purpura, Hepatitis C, Hereditary Spinal Ataxia, HIV, Huntington's Disease, Hypertension, Hyperventilation, Hypoglycemia, Impotence, Inflammatory Bowel Disease IBD, Lipomatosis, Lyme Disease, Lymphoma, Malignant Melanoma, Mania, Medical Marijuana Benefits, Melorheostosis, Meniere's Disease, Migraine Headache, Migraine Medical Marijuana, Motion Sickness, Mucopolysaccharidosis MPS, Multiple Sclerosis, Muscle Spasms, Muscular Dystrophy, Myeloid Leukemia, Nail-Patella Syndrome, Nightmares, Obesity, Obsessive Compulsive Disorder, Opiate Dependence, Osteoarthritis, Panic Disorder, Parkinson's Disease, Peripheral Neuropathy, Porphyria, Post Polio Syndrome PPS, Post Traumatic Stress Disorder, Post-traumatic arthritis, Premenstrual Syndrome PMS, Prostate Cancer, Prostatitis, Psoriasis, PTSD, Pulmonary Fibrosis, Quadriplegia,



#12

SECTION B AND C ATTACHMENT

An itch could be as debilitating as chronic pain

Published: June 4, 2013

ARTICLE BY: Dr. Murray Feingold, a Hazleton native, is on the staff of Boston Children's Hospital and Harvard Medical School. He is also the medical editor of CBS Boston Radio and writes a syndicated medical column.

Chronic pruritus can have a significant psychological impact resulting in anxiety and depression. Dermatological factors are due to conditions that primarily affect the skin. Examples would be eczema, psoriasis and contact dermatitis.

A variety of medications that work in different ways are used when the pruritus doesn't respond to first line therapies.

Unfortunately, at times, it may be difficult to find an effective medication.

An itch could be as debilitating as chronic pain

By DR. MURRAY FEINGOLD)

Published: June 4, 2013

How bad can a chronic itch be? According to one study it can be as debilitating as chronic pain.

Let's first define some terms. The medical word for itch is pruritus. Chronic pruritus is defined as an itch that lasts longer than six weeks.

The itch can be generalized, that is, affecting the entire body, or localized, affecting a specific area of the body such as the scalp or back.

A recent article discussed the complexity of chronic pruritus including why, at times, it is so debilitating.

Chronic pruritus can have a significant psychological impact resulting in anxiety and depression.

Frequently, the itching is so intense that it results in sleepless nights. Sleep deprivation affects a person's ability to function normally during the day.

Also, because of the intense itching, scratching takes place causing breaking of the skin followed by a secondary infection.

Chronic pruritus can be caused by dermatological and non-dermatological factors.

Dermatological factors are due to conditions that primarily affect the skin. Examples would be eczema, psoriasis and contact dermatitis.

There are many non-dermatological or systemic diseases that have pruritus as one of its symptoms. Examples include chronic kidney disease, gallbladder disease and hyperthyroidism.

There can also be psychological causes.

In order to treat chronic pruritus, its cause must first be determined. Diagnosis is usually easier if it is due to a skin condition than if it is secondary to a systemic disease or a non-skin condition.

If the itch is due to a systemic disease, the disease must first be treated in order to relieve the pruritus.

Numerous medications are available that can help relieve the itch.

Certain lotions or moisturizers are helpful but if the itching is intense, other medications such as antihistamines and steroids are usually given.

Frequently, stronger drugs are needed.

A variety of medications that work in different ways are used when the pruritus doesn't respond to first line therapies.

Unfortunately, at times, it may be difficult to find an effective medication.

Although chronic pruritus may not engender the worry and anxiety associated with cancer or heart disease, it still can have a significant deleterious effect on a person's life.

Dr. Murray Feingold, a Hazleton native, is on the staff of Boston Children's Hospital and Harvard Medical School. He is also the medical editor of CBS Boston Radio and writes a syndicated medical column.

#13

SECTION G Attachment

Cannabinoid Treatment: Itching (Pruritus)

Writing in the August 2002 issue of the American Journal of Gastroentrology, investigators from the *University of Miami Department of Medicine* reported successful treatment of pruritus with 5 mg of THC in three patients with cholestatic liver disease.[1] Prior to cannabinoid therapy, subjects had failed to respond to standard medications and had lost their ability to work.

Dermatology experts now believe that cannabinoids and the cannabinoid system may represent "promising new avenues for managing itch more effectively.

Itching (pruritus) is a common symptom associated with numerous skin diseases, as well as a secondary symptom of numerous serious conditions such as renal failure and liver disease. Itching, unlike other skin sensations, is generally a result of CNS activities and typically goes untreated by standard medical therapies.

A review of the scientific literature reveals three clinical trials investigating the use of cannabinoids in the treatment of pruritus. Writing in the August 2002 issue of the American Journal of Gastroentrology, investigators from the University of Miami Department of Medicine reported successful treatment of pruritus with 5 mg of THC in three patients with cholestatic liver disease.[1] Prior to cannabinoid therapy, subjects had failed to respond to standard medications and had lost their ability to work. Following evening cannabinoid administration, all three patients reported a decrease in pruritus, as well as "marked improvement" in sleep and were eventually able to return to work. Resolution of depression was also reported in two out of three subjects. "Delta-9-tetrahydrocannabinol may be an effective alternative in patients with intractable cholestatic pruritus," investigators concluded.

The following year, British researchers reported in the June 2003 issue of the journal *Inflammation Research* that the peripheral administration of the synthetic cannabinoid agonist HU-211 significantly reduced experimentally-induced itch in 12 subjects.[2] Investigators had previously reported that topical application of HU-210 on human skin reduced experimentally-induced pain and acute burning sensations.[3]

Most recently, researchers at Wroclaw, Poland's University of Medicine, Department of Dermatology, reported that application of an endocannabinoid-based topical cream reduced uremic pruritus and xerosis (abnormal dryness of the skin) in hemodialysis patients.[4] Three weeks of twice-daily application of the cream "completely eliminated" pruritus in 38 percent of trial subjects and "significantly reduced" itching in others. Eighty-one percent of patients reported a "complete reduction" in xerosis following cannabinoid therapy.

In light of these encouraging preliminary results, some dermatology experts now believe that cannabinoids and the cannabinoid system may represent "promising new avenues for managing itch more effectively."[5]

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[3] Dvorak et al. 2003. Cannabinoid agonists attenuate capsaicin-induced responses in human skin. *Pain* 102: 283-288.

[4] Szepietowski et al. 2005. Efficacy and tolerance of the cream containing structured physiological lipid endocannabinoids in the treatment of uremic pruritus: a preliminary study. *Acta Dermatovenerologic Croatica* (Croatia) 13: 97-103.

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#14

a report published in the American Journal of Medicine, which found a strong correlation between marijuana use and a smaller waistband. The study, conducted by researchers from the University of Nebraska, Harvard and Beth Israel Deaconess Medical Center, examined 4,657 adults, 579 of whom regularly smoked reefer.

"We found significant associations between marijuana use and smaller waist circumferences," the researchers report. Specifically, potheads had 16% lower levels of insulin than their non-smoking peers.

The hormone regulates metabolism of fat and carbs. The Atlantic noted that the findings perhaps explain why smokers of the wacky tobacky are less prone to diabetes. Even people who had smoked weed in the past but did not currently puff showed similar, though less striking, results.

Smoking Cannabis Reduces Pain, Helps Sleep And Improves Mood For Those With Chronic Symptoms

Our body contains a natural cannabinoid system that regulates health and illness

It wasn't until the mid-1990s that scientists discovered why marijuana works so well, and for so many different illnesses. The discovery was a natural system in the human body called the endocannabinoid system, which includes chemicals that mimic the activity of cannabis, called endocannabinoids.

Much like cannabis, Dr. Auld notes that endocannabinoids act to "decrease inflammation, increase immunity, decrease pain, and increase appetite."

cannabinoids have been found. CB1receptors are primarily located in the central nervous system and CB2 receptors are primarily found in the periphery of the body, especially in the immune system. CB1 receptors are located in diverse areas of the brain and are activated by endocannabinoids that are produced naturally in the nervous system and function to regulate various nervous system processes.

For patients with chronic (long-term) neuropathic pain, smoking cannabis was found to reduce symptoms of pain, improve mood and help sleep, a report published in *CMAJ* (Canadian Medical Journal Association) revealed. When damage or dysfunction of the nervous system results in chronic neuropathic pain, patients have few treatment options, such as antidepressants, local anesthetics, anticonvulsants or opioids. However, these medications often have undesirable side effects and do not work for everybody.

The authors in the article published in *CMAJ* inform that oral cannabinoids have been effective in reducing the symptoms of some types of pain. However, they many have different effects and risks compared to smoked cannabis.

Investigators from McGill University Health Centre (MUHC) and McGill University carried out a randomized, controlled trial to determine the analgesic effect of smoked cannabis in 21 patients, aged 18 years or more, all of them with chronic neuropathic pain. THC levels (drug potencies) were divided into 2.5%, 6% and 9.4%. Some participants also received a placebo (0%).

The researchers inform that there was a correlation between increased THC content and better sleep quality. Symptoms of depression and/or anxiety were also reduced at 9.5% THC level.

chemicals in marijuana, known as cannabinoids, actually mimic the activity of chemicals found naturally in the brain.

#15

Cannabinoids as Treatment for Psoriasis Symptoms

Research:

cannabinoids inhibit keratinocyte proliferation, and therefore support a potential role for cannabinoids in the treatment of psoriasis.

Cannabis might treat psoriasis, due to the anti-inflammatory properties of its cannabinoids, and the regulatory effects of THC on the immune system. The adverse effects of cannabis might be overcome by use of more specific cannabinoid receptor medications, to inhibit keratinocyte proliferation. Moreover, they observe that cannabinoids are readily absorbed through the skin. This is the ideal method of treating psoriasis, as it avoids some of the toxicity associated with systemic therapies.

The researchers tested the effects of four plant cannabinoids – Δ-9 tetrahydrocannabinol, cannabidiol, cannabinol and cannabigerol – on rapidly proliferating, cultured human keratinocytes.

All four cannabinoids inhibited keratinocyte growth in a dose-dependent manner, they report.

Despite varying degrees of affinity for cannabinoid receptors (CBs) among the substances tested, the extent of growth inhibition was similar with all four cannabinoids, implying a non-specific effect.

In confirmation of this, the investigators found that selective CB2 agonists only partially inhibited keratinocyte growth, while a non-selective CB agonist had a concentration-dependent effect.

Neither CB1 nor CB2 antagonists attenuated the effects of the CB agonists or the cannabinoids, however. Furthermore, these antagonists actually exhibited direct dose-dependent inhibition of keratinocyte growth.

Cannabinoids, as novel additions to the antipsoriatic ammunition.

Source

Dermatology Department, Shiraz University of Medical Sciences, Shiraz, Iran. namazi_mr@yahoo.com

cannabinoids, which exert inhibitory effects on antigen processing and macrophage/T-cell interaction and on the release of IL-2, TNF-alpha and nitric oxide from immune cells.

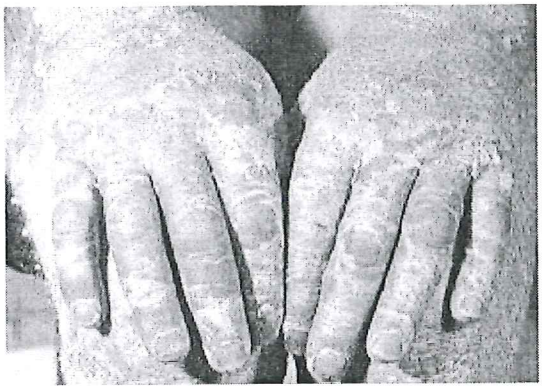
Testimonials:

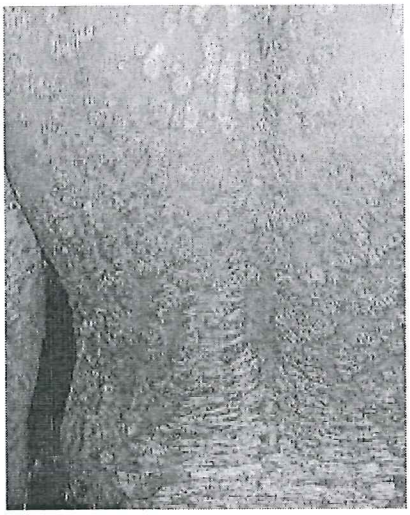
Cannabinoids extracted with grape seed oil: can be taken orally or topically applied or both.

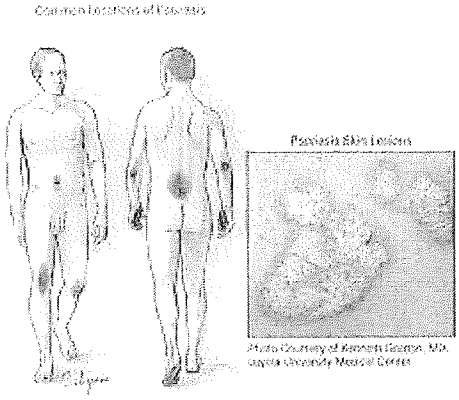
"Smoking marijuana keeps me from having flare-ups of psoriasis."

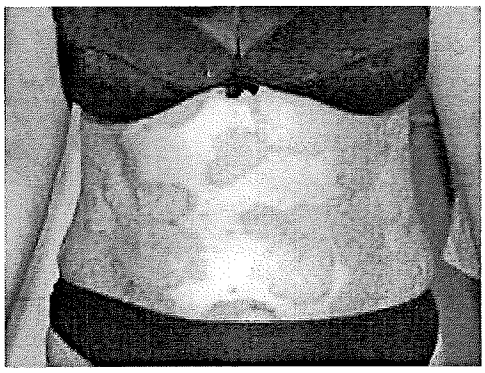
""HempEase" made from cannabis roots (no THC) has powerful skin treating qualities".

Cannabinoids can be taken orally, and applied topically for the treatment of psoriasis symptoms with little to no side effects.









Sativa x Indica hybrid

Side effects of psoriasis medication s (pharmaceuticals) include:
? Severe allergic reactions (rash; hives; itching; difficulty breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); black, tarry stools; blood in the urine; change in the appearance of a mole; chest pain; confusion; dark urine; diarrhea; fast or irregular heartbeat; flushing of the face, chest, back, or abdomen; gum disease or overgrowth; increased or decreased urination; loss of coordination; mental or mood changes; muscle cramps; numbness or tingling of the skin; seizures; severe or persistent headache or dizziness; shortness of breath; symptoms of infection (eg, chills, cough, fever, painful urination, sore throat); tremors; unusual bleeding or bruising; unusual lumps; unusual thickening or growth on the skin; unusual tiredness or weakness; vision changes; wheezing; yellowing of the skin or eyes, acne, dizziness, headache, increased hair growth, runny nose, sleeplessness, vomiting and stomach discomfort.

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4. Stern RS. Psoralen and ultraviolet a light therapy for psoriasis. N Engl J Med. 2007; 357(7):682-690. [PubMed]
Writing in the Journal of Dermatological Science, Jonathan Wilkinson (Nottingham University) and Elizabeth Williamson (Reading University) say that cannabinoids have anti-inflammatory properties and can inhibit the growth of cancer cell lines.

#16

In this day and age there is a large body of evidence that shows cannabis as an effective treatment for psoriasis and associated symptoms including sleep disorder and helps dampen stress and anxiety that causes flare-ups and as a result of the disease symptoms causing a vicious cycle. The medical Marijuana has proven itself to be a huge benefit for many; due to the anti-inflammatory properties of its cannabinoids that inhibit keratinocyte proliferation through a non-CB1/CB2 mechanism and has a therapeutic value in the treatment of psoriasis with their regulatory effects on the immune system while avoiding the toxicity associated with systemic therapies.

There is ample evidence that shows that activating the CB1 receptor, as triggered by THC in marijuana, inhibits the inflammation and cell proliferation that cause the disease's miserable and disfiguring side effects. CB2 receptors in a patient's epidermis have been proven to react very well to cannabinoids and leave skin feeling healthy and more improved.

The human body has naturally occurring endocannabinoids similar to THC that stimulate your cannabinoid receptors and produce a variety of important physiologic processes. Research has shown that our bodies are actually hard-wired to respond to cannabinoids through this unique cannabinoid receptor system; to date it's known that cannabinoid receptors play an important role in many body processes, including metabolic regulation, cravings, pain, anxiety, bone growth, and immune function.

A report by the American College of Physicians (ACP) further notes that:

"Marijuana has been smoked for its medicinal properties for centuries. It was in the U.S. Pharmacopoeia until 1942 when it was removed because federal legislation made the drug illegal ... Still, the overwhelming number of anecdotal reports on the therapeutic properties of marijuana sparks interest from scientists, health care providers, and patients. Dr. Frank Lucido, a Berkeley physician regularly recommends medical marijuana, said the plant's anti-inflammatory effects didn't surprise him. He has had patients who say their psoriasis, an immune disease that affects the skin and joints, and asthma get better when they smoke marijuana.

Over the past 20 years, researchers have discovered cannabinoid receptors: CB1, which mediates the central nervous system (CNS), and CB2, which occurs outside the CNS and is believed to have anti-inflammatory and immunosuppressive activity."

I will include proof of the medicinal help and benefit that marijuana can provide to those suffering from the chronic and debilitating effect of Psoriasis. (That information at the bottom) Cannabinoids can be taken orally, and applied topically for the treatment of psoriasis symptoms with little to no Side effects of psoriasis medication s (pharmaceuticals) included.

PeerJ. 2013 Feb 19; A novel control of human keratin expression: cannabinoid receptor 1-mediated signaling down-regulates the expression of keratins K6 and K16 in human keratinocytes in vitro and in situ.Ramot Y, Sugawara K, Zákány N, Tóth BI, Bíró T, Paus R.SourceDepartment of Dermatology, University of Luebeck , Luebeck , Germany ; Department of Dermatology, Hadassah-Hebrew University Medical Center , Jerusalem , Israel. Abstract: Cannabinoid receptors CB are expressed throughout human skin epithelium. CB1 activation inhibits human hair growth and decreases proliferation of

epidermal keratinocytes. Since psoriasis is a chronic hyperproliferative, inflammatory skin disease, it is conceivable that the therapeutic modulation of CB signaling, which can inhibit both proliferation and inflammation, could win a place in future psoriasis management. Given that psoriasis is characterized by up-regulation of keratins K6 and K16, we have investigated whether CB1 stimulation modulates their expression in human epidermis. Treatment of organ-cultured human skin with the CB1-specific agonist, arachidonoyl-chloro-ethanolamide ACEA, decreased K6 and K16 staining intensity in situ. At the gene and protein levels, ACEA also decreased K6 expression of cultured HaCaT keratinocytes, which show some similarities to psoriatic keratinocytes. These effects were partly antagonized by the CB1-specific antagonist, AM251. While CB1-mediated signaling also significantly inhibited human epidermal keratinocyte proliferation in situ, as shown by K6/Ki-67-double immunofluorescence, the inhibitory effect of ACEA on K6 expression in situ was independent of its anti-proliferative effect. Given recent appreciation of the role of K6 as a functionally important protein that regulates epithelial wound healing in mice, it is conceivable that the novel CB1-mediated regulation of keratin 6/16 revealed here also is relevant to wound healing. Taken together, our results suggest that cannabinoids and their receptors constitute a novel, clinically relevant control element of human K6 and K16 expression.

CMAJ. 2010 Oct 5;182(14):E694-701. doi: 10.1503/cmaj.091414. Epub 2010 Aug 30.

Smoked cannabis for chronic neuropathic pain: a randomized controlled trial.

A single inhalation of 25 mg of 9.4% tetrahydrocannabinol herbal cannabis three times daily for five days reduced the intensity of pain, improved sleep and was well tolerated

Source

Department of Anesthesia, McGill University, Montréal, Que. mark.ware@muhc.mcgill.ca

Source

Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461.

Examination of central nervous system tissue revealed a marked reduction of inflammation in the THC-treated animals. Therefore, as THC has been shown to inhibit both clinical and histologic EAE, it may prove to be a new and relatively innocuous agent for the treatment of immune-mediated diseases.

PMID: 2542370 [PubMed - indexed for MEDLINE]

Smoking marijuana several times a week leaves a lasting effect on a healthy person's immune system, a new study from Florida says. But this may actually boost opportunities for the medical use of marijuana.

Examination of central nervous system tissue revealed a marked reduction of inflammation in the THC-treated animals. Therefore, as THC has been shown to inhibit both clinical and histologic EAE, it may prove to be a new and relatively innocuous agent for the treatment of immune-mediated diseases.

PMID: 2542370 [PubMed - indexed for MEDLINE]

Writing in the Journal of Dermatological Science, Jonathan Wilkinson (Nottingham University) and Elizabeth Williamson (Reading University) say that cannabinoids have anti-inflammatory properties.

#17

Here is evidence that activating the CB1 receptor, as triggered by THC in marijuana, inhibits the inflammation and cell proliferation that cause the disease's miserable and disfiguring side effects.

PeerJ. 2013 Feb 19; .A novel control of human keratin expression: cannabinoid receptor 1-mediated signaling down-regulates the expression of keratins K6 and K16 in human keratinocytes in vitro and in situ.Ramot Y, Sugawara K, Zákány N, Tóth BI, Bíró T, Paus R.SourceDepartment of Dermatology, University of Luebeck , Luebeck , Germany ; Department of Dermatology, Hadassah-Hebrew University Medical Center , Jerusalem , Israel. Abstract: Cannabinoid receptors CB are expressed throughout human skin epithelium. CB1 activation inhibits human hair growth and decreases proliferation of epidermal keratinocytes. Since psoriasis is a chronic hyperproliferative, inflammatory skin disease, it is conceivable that the therapeutic modulation of CB signaling, which can inhibit both proliferation and inflammation, could win a place in future psoriasis management. Given that psoriasis is characterized by up-regulation of keratins K6 and K16, we have investigated whether CB1 stimulation modulates their expression in human epidermis. Treatment of organ-cultured human skin with the CB1-specific agonist, arachidonoyl-chloro-ethanolamide ACEA, decreased K6 and K16 staining intensity in situ. At the gene and protein levels, ACEA also decreased K6 expression of cultured HaCaT keratinocytes, which show some similarities to psoriatic keratinocytes. These effects were partly antagonized by the CB1-specific antagonist, AM251. While CB1-mediated signaling also significantly inhibited human epidermal keratinocyte proliferation in situ, as shown by K6/Ki-67-double immunofluorescence, the inhibitory effect of ACEA on K6 expression in situ was independent of its anti-proliferative effect. Given recent appreciation of the role of K6 as a functionally important protein that regulates epithelial wound healing in mice, it is conceivable that the novel CB1-mediated regulation of keratin 6/16 revealed here also is relevant to wound healing. Taken together, our results suggest that cannabinoids and their receptors constitute a novel, clinically relevant control element of human K6 and K16 expression.

via A novel control of human keratin expression: cannabino... [PeerJ. 2013] – PubMed – NCBI.

#18

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JOURNAL OF Dermatological Science

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Cannabinoids inhibit human keratinocyte proliferation through a non-CB1/CB2 mechanism and have a potential therapeutic value in the treatment of psoriasis

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Summary

Background

Cannabinoids from cannabis (*Cannabis sativa*) are anti-inflammatory and have inhibitory effects on the proliferation of a number of tumorigenic cell lines, some of which are mediated via cannabinoid receptors. Cannabinoid (CB) receptors are present in human skin and anandamide, an endogenous CB receptor ligand, inhibits epidermal keratinocyte differentiation. Psoriasis is an inflammatory disease also characterised in part by epidermal keratinocyte hyper-proliferation.

Objective

We investigated the plant cannabinoids Δ-9 tetrahydrocannabinol, cannabidiol, cannabinol and cannabigerol for their ability to inhibit the proliferation of a hyper-proliferating human keratinocyte cell line and for any involvement of cannabinoid receptors.

Methods

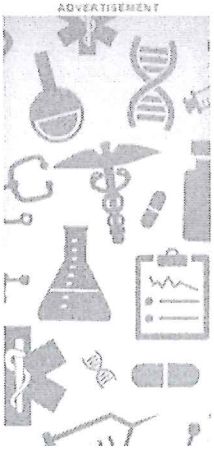
A keratinocyte proliferation assay was used to assess the effect of treatment with cannabinoids. Cell integrity and metabolic competence confirmed using lactate-dehydrogenase and adenosine tri-phosphate assays. To determine the involvement of the receptors, specific agonist and antagonist were used in conjunction with some phytocannabinoids. Western blot and RT-PCR analysis confirmed presence of CB1 and CB2 receptors.

Results

The cannabinoids tested all inhibited keratinocyte proliferation in a concentration-dependent manner. The selective CB2 receptor agonists JWH015 and BML190 elicited only partial inhibition, the non-selective CB agonist HU210 produced a concentration-dependent response, the activity of theses agonists were not blocked by either CB1/CB2 antagonists.

Conclusion

The results indicate that while CB receptors may have a circumstantial role in keratinocyte proliferation, they do not contribute significantly to this process. Our results show that cannabinoids inhibit keratinocyte proliferation, and therefore support a potential role for cannabinoids in the treatment of psoriasis.



Section G & Section H Attachment

#19

The purpose of this document and all attached and or accompanying supporting documents is for the purpose of including Psoriasis and Psoriatic Arthritis to the List of Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.

Section G: Attachment – General Evidence of Support for Medical Marijuana

" H "

Dr. Frank Lucido, a Berkeley physician who regularly recommends medical marijuana, said the plant's anti-inflammatory effects didn't surprise him. He has had patients who say their psoriasis, an immune disease that affects the skin and joints, and asthma get better when they smoke marijuana.

See page 3 of his attached report for confirmation

SEE PAGE 3

(1)

Medical Marijuana In California, 1996-2006

Frank Lucido, MD *(Berkley) is

a family practitioner who began approving patients' use of cannabis soon after the passage of Prop. 215. He conducts about 900 cannabis consultations per year (including follow-up visits)

1. Approvals issued: >3,000
2. Previously self-medicating: 99%
3. Conditions treated with cannabis:

Chronic pain 75% Most diagnoses are musculoskeletal, i.e. dis disease, post-traumatic injuries, etc. Others include Fibromyalgia Syndrome (2%), Rheumatoid Arthritis, Psoriatic Arthritis, Systemic Lupus Erythematosis, Lyme Arthritis, Raynaud's Syndrome, Gout, Gulf War Syndrome.

*Psychiatric problems (33%),

including Depression, Chronic Anxiety,
Insomnia, Bipolar, PTSD, ADHD, and OCD. Neurologic problems (14%), including headache, Multiple Sclerosis, Restless Leg Syndrome, Parkinson's, Neuropathic Pain, Tremor, Seizure Disorder.

*Genito-Urinary problems (5.6%),

including sever Dismenorrhea, Menopausal Syndrome, Endometriosis, PMS, Interstitial Cystitis, Nephrolithiasis

*Gastrointestinal (12.5%),

including chronic abdominal pain, hepatitis C, Irritable Bowel Syndrome, Crohn's Ulcerative Colitis, GERD, Anorexia, Nausea, Diverticulitis pain.

*Others (11%), notably Glaucoma (3%),

Medication side effects (2.6%), Asthma (2.3%),

Cancer, lymphoma, leukemia, Meniere's/tinnitus.

Effects of cannabis:
Patients are able to be more active (work, exercise, etc.); sleep, eating and overall ability to function improved.g

Drug use reduced?
Chronic pain patients report reduced use of opioids, NSAIDs, muscle relaxants, sleeping pills.
Psychiatric and insomnia patients reduce use of tranquilizers, SSRT antidepressants, and sleeping

pills. Neurologic patients reduce use of opioids, muscle relaxants, NSAIDs, tiptans and other migraine headache remedies.

Unusual conditions treated?
Gulf War Syndrome. Patients use cannabis to mitigate chronic neuropathic pain form nerve damage, chronic nausea, and migraine, as well as PTSD from his experiences in combat.

Comments re strains and dosage:
Patients vary tremendously in their dosage needs. In general, Sativa strains seem to have a mildly stimulating effect and are best for daytime use. Indica has a mildly sedative effect and is best for evening use. Both are reportedly effective for chronic pain.

Adverse effects?
Reported adverse effects are rare, in part because the patients coming to a medical cannabis consultation has already found cannabis to be of benefit. I have had perhaps 10 patients in 10 years who had never tried cannabis or who hadn't use it in many years and were uncertain if it would effectively treat their current illness or symptoms. Two patients have discontinued use in response to decreased productivity. The overwhelming majority report that they are MORE productive when their symptoms are controlled with cannabis.

Demographics

In a recent series of 393 consecutive patients, 195 were male, 108 female.
Their ages, plotted, would form a bell curve:

<18	2	30-39	60	60-69	24
18-20	4	40-49	80	70-79	1
21-29	42	50-59	89	80-89	1

ADHD patients?
Over the course of my practice I have approved cannabis use by about five patients per year for attention deficit disorders. In recent years, however, more patients report using cannabis to treat ADD and AD/HS, and I am issuing more approvals. The present rate is approximately one patient per month.

Cannabis as a substitute for alcohol?
I have had only three patients in 10 years whose primary diagnosis for cannabis was alcoholism. Many recovering alcoholics are using cannabis for chronic anxiety and/or depression, so to some extent they are substituting it as a treatment for problems they previously self-treated with alcohol. Because alcohol can damage the liver and cause destructive behavior, cannabis use is rightly termed "harm reduction"

1. Approvals issued: 12,000
2. Previously self-medicating: 80%
3. Conditions being treated:

Chronic pain, 85%.
Includes all etiologies from systemic disease, i.e., Fibromyalgia, Lupus, Rheumatlid Arthritis, to physical injuries such as fractures incurred in motor vehicle accidents, gunshot wounds, failed surgeries, Post-Traumatic Arthritis, Osteoarthritis and work-related problems.

Psychiatric disorders, 15%. Includes *PTSD*,
Depression, Anxiety, Insomnia, Panic disorder. Other illnesses include AIDS and Cancer (2%-3%), Glaucoma (1%-2%). Psoriasis and Eczema (1%-2%). Patients report feeling better able to face whatever illness they are
dealing with.

Results reported:
The majority of my patients report a decrease in the use of conventional pharmaceuticals. Approximately 90% of those using narcotics decrease their usage, and about half discontinue them altogether. Patients report feeling better able to face whatever illness they are dealing with. Many express relief that their pain and anxieties are being treated through a God-given plant. Cannabis enables them to feel a part of their own treatment and a part of their own healing.

Health is a state of mind, body and spirit. By restoring their connection to nature, cannabis helps patients on all three levels. *Medications discontinued or reduced *include Oxycontin, Norco, Percoset, Vicodin, Flexeril, Soma, Valium, SSRI antidepressants, and blood-pressure medications Norvasc and Hydrochlorothiazide.

Approximately 1% of my patients report reduced reliance or discontinuation of seizure medication by substituting Cannabis for Dilantin and remain seizure free. Many of my Glaucoma patients no longer require their timoptic drops and are able to maintain normal pressures with the use of Cannabis.

Many of my patients who have lost hope in conventional pharmaceutical treatments report enhanced health, decreased pain, decreased depression and an overall sense of well-being despite chronic illness.

Unusual conditions successfully treated with cannabis?

Eczema, Psoriasis, and dermatits or all types are being treated successfully. Also, skin reactions associated with Agent Orange. Although it is not a medical condition per se, parenting problems are alleviated by the use of cannabis. Mothers and fathers report enhanced flexibility and an ability to identify the child's needs as those of a separate and unique individual. Parents are able to interpret the child's behavior in an age-appropriate manner. Improved communication leads to shared experience. The parent becomes present and the child benefits from the increase positive attention. Many patients report that cannabis stimulates their interest in art, music, poetry, writing, and other creative endeavors. Insight is manifested by an ability to recognize one's place in the universe. Patients say cannabis makes them less self-centered and egocentric and more aware of the needs of other people. It makes them aware of how their own behavior affects other people and how they may be contributing to negative interaction. Cannabis can be useful adjunct in the marital-counseling process.

Comments re strains and dosage?
As a result of Prohibition, not enough information is available regarding strains and I don't feel comfortable making a comment on this subject. Regarding delivery methods, I feel strongly that edible cannabis is underutilized. As noted in a previous communication (Spring 2006), oral ingestion involves processing by the liver, which minimizes the differences between strains. Oral ingestion is recommended for those seeking long term relief from chronic physiological problems such as pain, glaucoma, diabetes, lupus, rheumatoid arthritis, and multiple sclerosis.

Just as patients who smoke cannabis learn to inhale as needed to achieve and maintain their desired effect, patients who use oral cannabis can employ an analogous titration process. If a patient is using a vegetable-oil extract, s/he calculates the amount needed top produce the desired effect without over-sedation (the "loading dose").

By determining how long it takes for the effect to come on and wear off, patients can schedule a subsequent"maintenance dose" to keep on an even keel. The sedation that may be perceived as a negative side effect during waking hours is precisely the effect that chronic pain patients and others require for a good nit's sleep.

Orally ingested cannabinoids can exert their effects for close to eight hours - adequate sleep for most patients- eliminating the need for a maintenance dose in the middle of the night. The efficacy of cannabis applied topically as an ointment or tincture is similarly underrated. Dose is controlled by the individual monitoring the
effects on the skin lesions being treated. My patients have had great success with using 1/4 cup of extracted cannabis oil in a hot bath for overall distribution, followed by localized applications to severely inflamed areas.

Cannabinoids and possibly other healing components of the plant are absorbed directly through the skin; the anti-inflammatory properties are outstanding, reducing recovery time from injuries and promoting healing of lesions. Topical cannabis has also been used by my lupus patients and rheumatoid arthritis patients to increase the function of joints and decrease nodule formation. Many recipes are available both vegetable-oil-based and rubbing-alcohol-based preparations.

Adverse effects?
The most significant negative reactions are due to fear of incarceration and
the results of abuse by officers unwilling to honor California law.

Demographics

My office does not compile this data, but I can generalize with some assurance that my patients are about two-thirds male and more than half are over 50 years old. They are predominately white, with the majority having completed high school and beyond. Those patients who are not disabled do not report problems getting and maintaining satisfactory employment. Most use Cannabis in the evening for relaxing and for chronic stresses and pains associated with the workday and are not under the influence during work hours.

ADHD patients?
ADHD diagnoses are misleadingly low. There are many high-achieving successful, adults who use cannabis for other problems but in fact meet the criteria for an ADHD diagnosis. Most ADHD patients in my practice are teenagers with parental consent to substitute Cannabis for more dangerous and addicting drugs like Ritalin, Dexedrine, etc. These patients do much better with Cannabis, show marked improvement in appetite and sleep, and are more successful in school.

Substitute for alcohol?
More that half my patients express a preference for Cannabis over alcohol. Those who have been alcoholics as evidenced by DUI and other court proceedings find that substituting Cannabis for alcohol makes it much easier to remain sober. Ample research demonstrates the excessive alcohol use often results in domestic violence and motor vehicle accidents. This is not the case with Cannabis use form my experience.

Philip A Denney, MD (Redding, Lake Forest, Carmichael),
spent most of his career as a family practitioner before specializing in cannabis consultations in 1999. Aware that patients from all over the state were coming to see him in an office near Sacramento, Denney expanded his practice in 2004, opening offices in Orange and Shasta counties (in partnership with Robert Sullivan, MD, whose separate response is on page 8)).

Approvals issued: 18,900

Previously self-medicating: 95%

Conditions being treated:

Chronic pain 50% (trauma, surgical, neuropathic, etc.) Cannabis works particularly well for neuropathic pain.

Gastrointestinal conditions 15% (nausea, vomiting, Crohn's disease, hepatitis C, etc.).

Psychiatric conditions 15% (anxiety, depression, bipolar disorder, PTSD, etc.).

Neurologic disorders 10% (multiple sclerosis, plegias, phantom pain, migraine, etc.).

Others 10%. Most common among these are glaucoma, addiction, and sleep disorders.

Results reported:* Cannabis is nontoxic and therefore quite safe. Dosing is easy, involving self-titration, and there is no "hangover" effect. We do not see any dependence or abuse problems.

Medications reduced:
Cannabis allows significant decreased use or elimination of many prescription medications, particularly narcotics. Patients usually report decreases of 50% or better.

Rare conditions being treated?
Many, including anorexia and other eating disorders, and rare cancers such as pheochromocytoma. I am particularly impressed with the usefulness of cannabis in Tourette's Syndrome.

Comments re strains and dosage?
There are virtually no pure Sativa or Indica strains being used by California patients because of long term crossbreeding. Nor can we subject strains in use to chemical analysis to determine their components.

The Indica type is preferred by patients for pain, spasm, sleep, and mania. Indicas are said to have a higher CBD-to-THC ratio than Sativas.

The Sativa type is reportedly better for appetite and to alleviate gastrointestinal symptoms. It has mild stimulant effects, elevating mood and increasing activity.

The Indica type is preferred by patients for pain, spas, sleep, and mania.

Indicas are said to have a higher CBD-to-THC ratio than Sativas.

Dosage varies widely. Approximately 80% of patients use one ounce per week or less; 20% use more. Patients ingesting via edibles or teas tend to use more. The highest use among my patients is three ounces per week.

Demographics data:
Not kept. Average age approximately 40 years. 75% male, 25% female.

Approval for ADHD?
A relatively common diagnosis, particularly in younger males.

Cannabis as a substitute for alcohol?
Patients frequently report success in using cannabis to maintain sobriety.
It is also used by many as a substitute for opiates and stimulants.
Cannabis is an underused treatment for substance abuse.*

Overdose from edible cannabis -an unpleasant drowsiness lasting six to eight hours- is rare and transient.*

Adverse effects?
Virtually none reported by patients except contacts with the legal system. Patients are able to stop using easily in order to pass drug tests or when traveling. Overdose form edible cannabis - an unpleasant drowsiness lasting 6-8 hours- is rare and transient.

#20

Sections G & H Attachment

The purpose of this document and all attached and or accompanying supporting documents is for the purpose of including Psoriasis and Psoriatic Arthritis to the List of Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.

Section G: Attachment – General Evidence of Support for Medical Marijuana

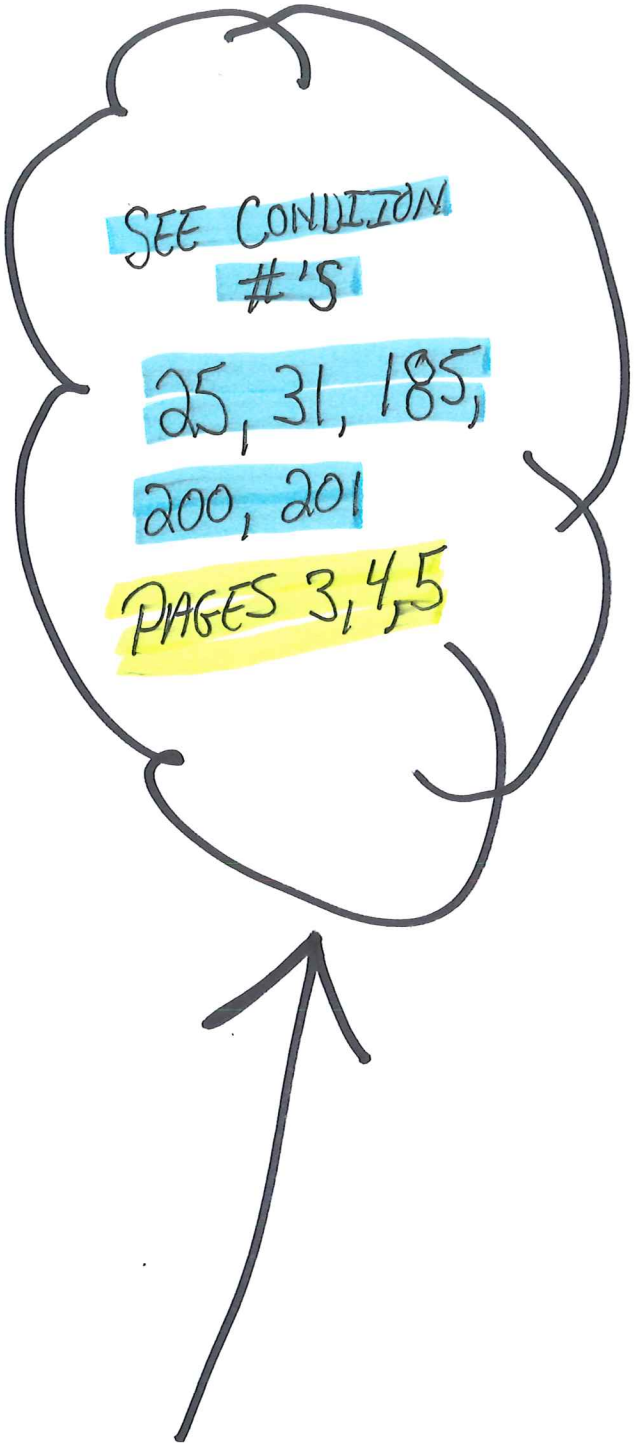
" H "

Dr. Tod Mikuriya, MD a psychiatrist and addiction medicine specialist; from 1966 to 1967, Dr. Mikuriya directed the drug addiction treatment center of the New Jersey Neuropsychiatric Institute, in Princeton. In 1967, he became a consulting research psychiatrist at the Center for Narcotics and Drug Abuse Studies of the National Institute of Mental Health, where he was in charge of marijuana research. He was an architect of Proposition 215, the state ballot measure that in 1996 made it legal for California doctors to recommend marijuana for seriously ill patients. He was also a founder of the California Cannabis Research Medical Group and its offshoot, the Society of Cannabis Clinicians.

See *page 3, number 31 (*Autoimmune Disease) and Page *5, numbers 200 & 201* (Psoriaias and Psoriatic Arthritis) of the attached report referencing a list of 259 conditions which he believed could be treated with Medical Cannabis.

See Page 4 number185 (Insomnia) also a problem for Psoriasis suffers.

The purpose of this document and all attached and or accompanying supporting documents is for the purpose of including Psoriasis and Psoriatic Arthritis to the List of Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.



Section G Attachment: General Evidence of Supportfor Medical Marijuana Treatment

259 Medical Conditions

Tod Mikuriya, MD a psychiatrist and addiction medicine specialist, posted a list of 259 conditions which he believed could be treated with medical cannabis. The report titled "International Classification of Diseases 9-CM 1996, Chronic Conditions Treated with Cannabis, Encountered Between 1990-2004" was first published online in 2001 and then updated in 2004.
Chronic inflammatory conditions respond well to cannabis compared with other analgesics.
The "ICD-9" numbers referenced in the list below are from the International Statistical Classification of Diseases and Related Health Problems published by the World Health Organization. ICD stands for International Classification of Diseases, and the number 9 refers to the 9th revision of that classification.

CONDITION	ICD-9 #	CONDITION	ICD-9 #
1. Acquired hypothyroidism	244	131. Major Depression, Recur	296.3
2. Acute Gastritis	535.0	132. Major Depression. Sgl Epis	296.2
3. Acute Sinusitis	461.9	133. Malignant Melanoma	172.9
4. ADD other	314.8	134. Mania	296.0
5. ADD w/hyperactivity	314.01	135. Marfan Syndrome	759.82
6. ADD w/o hyperactivity	314.0	136. Mastocytosis	757.33

7. Adrenal Cortical Cancer	194.0	137. Melorheostosis	739.99
8. Agoraphobia	300.22	138. Meniere's Disease	386.00
9. AIDS Related Illness	042	139. Menopausal syndrome	627.2
10. Alcohol Abuse [1]	305.0	140. Migraine(s)[1]	346.x
11. Alcoholism[1]	303.0	141. Migraine, Classical[1]	346.0
12. Alopecia	704.0x	142. Mononeuritis lower limb	355
13. Amphetamine Dependence	304.4	143. Motion Sickness	994.6
14. Amyloidosis	277.3	144. Mucopolysaccharoidosis	277
15. Amytrophic Lateral Sclerosis	335.2	145. Multiple joints pain	719.49
16. Anaphylactic or Reaction	995.0	146. Multiple Sclerosis	340.0
17. Angina pectoris	413	147. Muscle Spasm	728.85
18. Ankylosis	718.5	148. Muscular dystrophies	359
19. Anorexia Nervosa[1]	307.1	149. Myeloid leukemia	205
20. Anorexia[1]	783.0	150. Myofacial Pain Syndrome[3]	782.0
21. Anxiety Disorder[1]	300.00	151. Nail patella syndrome[1]	756.89
22. Arteriosclerotic Heart Disease	414.	152. Nausea	787.02
23. Arthritis, Degenerative	715.0	153. Nephritis/nephropathy	583.81
24. Arthritis, post	716.1	154. Neurasthenia	300.5

traumatic[1]			
25. Arthritis, Rheumatoid[1]	714.0	155. Neuropathy	357
26. Arthropathy, Degenerative[1]	716.9	156. Nightmares	307.47
27. Arthropathy, gout	274.0	157. Nonpsychotic Org Bra Dis[1]	310.8
28. Asthma, unspecified	493.9	158. Nystagmus, Congenital	379.50
29. Atrophy Blanche	701.3	159. Obesity, exogenous[1]	278.00
30. Autism/Aspergers	299.0	160. Obesity, morbid[1]	278.01
31. Autoimmune Disease	279.4	161. Obsessive Compulsive Disorder	300.3
32. Back Sprain	847.9	162. Opiate Dependence	304.0
33. Bell's palsy	351.0	162. Optic neuritis	377.30
34. Bipolar Disorder	296.6	164. Org. Mental Dis.hd inj	310.1
35. Brain malignant tumor	191	165. Osgood-Schlatter	732.4
36. Brain Trauma	310.9	166. Osteogenesis imperfecta	756.51
37. Bruxism	306.8	167. Other arthropod borne dis	088.
38. Bulemia	307.51	168. Other CNS demyelinating	341.
39. Cachexia	799.4	169. Other Skin Cancer[1]	173
40. Cancer, site unspecified	199	170. Other spinal cord disease	336

41. Cardiac conduction disorder	426	148. Pain, Ureter	788.0
42. Carpal Tunnel Syndrome	354.0	172. Pain, Vaginal	625.9
43. Cerebellar Ataxia	334.4	173. Pancreatitis	577.1
44. Cerebral Aneurism[1]	747.81	174. Panic Disorder[1]	300.01
45. Cerebral Palsy[1]	343.9	175. Paralysis, unspecified	344.9
46. Cervical Disk Disease	722.91	176. Paraplegia(s)	344.1x
47. Cervicobrachial Syndrome	723.3	177. Parkinsons Disease	332.0
48. Charcot-Marie-Tooth	356.1	178. Paroxysmal Atrial Tachy[3]	427.0
49. Chemotherapy Convales	V66.2	179. Patellar chondromalacia	717.7
50. Chronic Fatigue Syndrome	780.7	180. Pelvic Inflammatory Dis	614
51. Chronic Obst Pulmo Dis	491.90	181. Pemphigus	694.4
52. Chronic Sinusitis	473.9	182. Peptic Ulcer/Dyspepsia	536.8
53. Cluster Headaches	346.2	183. Peripheral enthesopathies	726
54. Cocaine Dependence[1]	304.2	184. Peritoneal Pain	726
55. Colitis Ulcerative	536.9	185. Persistent Insomnia	307.42
56. Colitis [1]	558.9	186. Peutz-Jehgers Syndrome[3]	756.9
57. Colon diverticulitis	562.1	187. Pneumothorax, Spontaneo	512.8

58. Color Blindness[2]	368.55	188. Polyarteritis Nodosa	446.0
59. Compression of Brain	348.4	164. Porphyria	277.1
60. Conjunctivitis	372.9	190. Post Cardiotomy Syndrome	429.4
61. Constipation	564.0	191. Post Concussion Syndrome	310.2
62. Cough[1]	786.2	192. Post Polio Syndrome	138.0
63. Cystic Fibrosis	518.89	193. Post Traumatic Stress Dis.	309.81
64. Darier's Disease [1]	291.0	194. Post W.E. Encephalitis	062.1
65. Delerium Tremens [1]	291.0	195. Premenstrual Syndrome[1]	625.3
66. Dentofacial anomaly pain	524.	196. Prostate Cancer	186
67. Dermatomyositis	710.3	197. Prostatis	600.0
68. Diabetes Adult Ons Unctrl	250.2	198. Pruritus, pruritic[1]	689.9
69. Diabetes Adult Onset 250.0	250.0	199. Pschogenic PAT	316.0
70. Diabetes Insulin Depend.	250.1	200. Psoriasis	696.1
71. Diabetic Neurpathy	250.6	201. Psoriatic Arthritis	696.0
72. Diabetic Ophthalmic Dis.	250.5	202. Psychogenic Dysuria	306.53
73. Diabetic PeripheralVascD	250.7	203. Pyschogenic Hyperhidrosis	306.3
74. Diabetic Renal Disease	250.4	204. Psychogenic Pain	307.89

75. Diarrhea	787.91	205. Psychogenic Pylorospasm[3]	306.4
76. Drusen of Optic Nerve	377.21	206. Pulmonary Fibrosis	516.3
77. Dumping Syndrome Post Sur	564.2	207. Pylorospasm Reflux	537.81
78. Dupuytens Contracture	728.6	208. Quadriplegia(s)	344.0x
79. Dyslexic Amblyopia[3]	368.0	209. Radiation Therapy	E929.9
80. Dysthymic Disorder	300.4	210. Raynaud's Disease	443.0
81. Eczema	692.9	211. Reflex Sympath Dystrophy	337.2
82. Ehlers Danlos Syndrome	756.83	212. Regional Enteritis & Crohns	555.9
83. Emphysema[3]	492.8	213. Reiters Syndrome	99.3
84. Endometriosis[3]	617.9	214. Restless Leg Syndrome	333.99
85. Eosinophilia-Myalgia Syn	710.5	215. Rosacea	695.3
86. Epidermolysis Bullosa	694.9	216. Schizoaffective Disorder	295.7
87. Epididymitis[3]	604.xx	217. Schizophrenia(s)	295.x
88. Epileysy(ies)[1]	345.x	218. Scleroderma	710.1
89. Erythma Multiforma	695.1	219. Scoliosis	754.2
90. Felty's Syndrome	714.1	220. Sedative Dependence[1]	304.1
91. Fibromyagia/Fibrositis	729.1	221. Senile Dementia[1]	290.0
92. Fore Arm/Wrist/Hand	959.3	222. Shingles (Herpes Zoster)	053.9

93. Friedreich's Ataxia	334.0	223. Shoulder Injury Unspec	959.2
94. Gastritis[1]	535.5	224. Sleep Apnea	780.57
95. GastroEsophgeal Rflx Dis	054.10	225. 192. Spina Bifida Occulta	756.17
96. Genital Herpes	054.10	226. Spinal mm atrophy II	335.11
97. Glaucoma	365.23	227. Spinal Stenosis	724.02
98. Glioblastoma Multiforme	191.9	228. Spondylolisthesis[3]	738.4
99. Grand Mal Seizures[3]	345.1	229. Strabismus & other binoc	378
100. Graves Disease[3]	242.0	230. Sturge-Weber Disease	759.6
101. Hemiparesis/plegia	342	231. Sturge-Weber Eye Syn[3]	759.6
102. Hemophilia A	286.0	231. Stuttering[2]	307.0
103. Henoch-Schoelein Purpur[4]	287.0	233. Syringomyelia	336.0
104. Hepatitis-non-viral	571.4	234. T.M.J. Syndrome	524.60
105. Herpetic infection of penis	054.13	235. Tenosynovitis	727.x
106. Hiccough[1]	786.8	237. Tension Headache	307.81
107. Hip	959.6	237. Testicular Cancer	186.9
108. Huntingtons Disease[1]	333.4	238. Testicular Torsion	608.2
109. Hypertension[1]	401.1	239. Thoracic Outlet Synd	353.0
110. Hyperventilation	786.01	240. Thromboangiitis Obliteran	443.1

111. Hypoglycemia(s)	251	241. Thyroiditis	245
112. Impotence, Psychogenic	302.72	242. Tic disorder unspec	307.20
113. Insomnia[1]	780.52	243. Tic Doloroux[1]	350.1
114. Intermittent Explosive Dis	312.34	244. Tietze's Syndrome	733.6
115. Intervertebral Disk Disease	722.x	245. Tinnitus	388.3
116. Irritable Bowel Synd	564.1	246. Tobacco Dependence	305.1
117. Ischemic Heart Disease	411.x	247. Tourette's Syndrome	307.23
118. IVDD Cerv w Myelopathy	722.71	248. Trachoria Growths[4] & 6	???.?
119. Jacksonian Epilepsy[3]	345.5	249. Tremor/Invol Movements	781.0
120. Knee, ankle & foot injury	959.7	250. Trichotillomania	312.39
121. Limbic Rage Syndrome[3]	345.4	251. Ureter spasm calculus	592
122. Lipomatosis	272.8	252. Urethritis/Cystitis	595.3
123. Lower Back Pain	724.5	253. Uterine cancer	236.0
124. L-S disk dis sciatic N irrit	722.1	254. Vertebral disloc unspec	839.4
125. Lumbosacral Back Disease	724.x	255. Viral B Hepatitis, chronic	070.52
126. Lupus	710.0	256. Viral C Hepatitis, chronic	070.54
127. Lyme Disease	088.81	257. Vomiting	787.01

128. Lympho & reticular ca	200	258. Whiplash	847.0
129. Lymphoma	2038.7	259. Writers' Cramp[5]	300.89
130. Macular Degeneration[3]	362.5		

Notations from Dr. Mikuriya's list:

1 = Represents citations fromn pre-1937 medical literature
2 = From Eugene Schoenfeld, MD
3 = From Dale Gieringer, PhD, California NORML
4 = From Robert Wilson, Hayward Hempery
5 = From Barry R. McCaffrey (12/30/96)
6 = Uncodeable. Possibly a specious disease submitted by an undercover agent.

Section G & H Attachment

#21

SECTION G ATTACHMENT
&
SECTION H

This document published 20 January 2011 in the *Journal of Investigative Dermatology* illustrates the clinical relevance and medical benefits derived by the therapeutic efficacy of cannabis which thereby warrants including Psoriasis to the List of chronic Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program

Endocannabinoids Modulate Human Epidermal Keratinocyte Proliferation and Survival via the Sequential Engagement of Cannabinoid Receptor-1 and Transient Receptor Potential Vanilloid-1

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We have recently shown that lipid mediators of the emerging endocannabinoid system (ECS) are key players of growth control of the human pilosebaceous unit. In this study, we asked whether the prototypic endocannabinoid anandamide (*N*-arachidonoylethanolamine, AEA) has a role in growth and survival of epidermal keratinocytes (KCs). Using human cultured KCs and skin organ-culture models, and by employing combined pharmacological and molecular approaches, we provide early evidence that AEA markedly suppresses KC proliferation and induces cell death, both *in vitro* and *in situ*. Moreover, we present that these cellular actions are mediated by a most probably constitutively active signaling mechanism that involves the activation of the metabotropic cannabinoid receptor CB_1 and a sequential engagement of the "ionotropic cannabinoid receptor" transient receptor potential vanilloid-1 (TRPV1). Finally, we demonstrate that the cellular effects of AEA are most probably due to a Ca^{2+} influx via the non-selective, highly Ca^{2+}-permeable ion channel TRPV1, and the concomitant elevation of intracellular Ca^{2+} concentration. The data reported here may encourage one to explore whether the targeted manipulation of the above signaling pathway of the cutaneous ECS could become a useful adjunct treatment strategy for hyperproliferative human dermatoses such as psoriasis or KC-derived skin tumors.

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INTRODUCTION

The emerging endocannabinoid system (ECS, Mechoulam *et al.*, 1998; Howlett *et al.*, 2002; Pacher *et al.*, 2006; Di Marzo, 2008) has lately been identified in the skin. Indeed, several human skin cell compartments produce prototypic endocannabinoids such as anandamide (*N*-arachidonoylethanolamine, AEA) and 2-arachidonoylglycerol, and express enzymes involved in the synthesis and metabolism of these lipid mediators (Calignano *et al.*, 1998; Berdyshev *et al.*, 2000; Maccarrone *et al.*, 2003; Karsak *et al.*, 2007). Furthermore, the G-protein-coupled metabotropic cannabinoid receptors (CB_1 and CB_2; Pertwee, 2005; Howlett, 2005; Mackie, 2006) as well as, to our knowledge, previously unreported, "ionotropic cannabinoid receptors" (such as transient receptor potential vanilloid-1, TRPV1; Di Marzo *et al.*, 1998, 2001; Zygmunt *et al.*, 1999) were identified, both *in situ* and *in vitro*, on numerous skin cell populations such as epidermal and hair follicle keratinocytes (KCs) and sebaceous gland-derived sebocytes (Casanova *et al.*, 2003; Bodó *et al.*, 2005; Ibrahim *et al.*, 2005; Stander *et al.*, 2005; Blazquez *et al.*, 2006; Karsak *et al.*, 2007; Telek *et al.*, 2007; Dobrosi *et al.*, 2008; Tóth *et al.*, 2009). These discoveries have made the ECS a topic of major interest in cutaneous neuroendocrinology and neuropharmacology (Bíró *et al.*, 2009; Kupczyk *et al.*, 2009).

The ECS is profoundly involved in the regulation of human epidermal homeostasis. Indeed, AEA inhibited the differentiation of cultured normal human epidermal KCs (NHEKs) and HaCaT KCs whose effect was mediated by increasing DNA methylation through mitogen-activated protein kinase-dependent pathways (p38, p42/44) triggered

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Abbreviations: AEA, N-arachidonoylethanolamine; $[Ca^{2+}]_i$, intracellular Ca^{2+} concentration; CAPS, capsaicin; ECS, endocannabinoid system; FLIPR, fluorescence image plate reader; G6PD, glucose-6-phosphate dehydrogenase; KC, keratinocyte; NHEK, normal human epidermal KC; RNAi, RNA interference; TRPV1, transient receptor potential vanilloid-1; TTP, time to peak

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by CB_1 activation (Maccarrone *et al.*, 2003; Paradisi *et al.*, 2008). Involvement of CB_1 in the regulation of epidermal differentiation is also suggested by the differential *in situ* expression of CB_1 in the human epidermis, with it being higher in the more differentiated (granular and spinous) layers (Casanova *et al.*, 2003; Stander *et al.*, 2005).

Published data on CB-coupled mechanisms in the regulation of epidermal KC proliferation appear to be conflicting. For example, phytocannabinoids (for example, Δ^9-tetrahydrocannabinol) derived from the plant *Cannabis sativa* as well as synthetic CB agonists inhibited growth of cultured transformed (HPV-16 E6/E7) human epidermal KCs; yet, these effects were $CB_{1/2}$ independent (Wilkinson and Williamson, 2007). In contrast, on tumorigenic transformed murine KCs (PDV.C57 and HaCa4), the growth-inhibitory actions of synthetic CB agonists were prevented by both CB_1 and CB_2 antagonists (Casanova *et al.*, 2003). Interestingly, synthetic CB_1 and CB_2 agonists were reportedly ineffective in modulating cellular growth of both cultured NHEKs and non-tumorigenic human (HaCaT) and murine (MCA3D) KCs (Casanova *et al.*, 2003).

However, none of the above studies have investigated the role of endocannabinoids in the regulation of human epidermal KC proliferation. Therefore, in this study, we have investigated the actions of the most extensively studied endocannabinoid, AEA, on the biology of human KCs. Our specific aims were to (i) define the *in vitro* and *in situ* effects of AEA on epidermal KC growth and survival; and (ii) identify those intracellular signaling pathways that may mediate the actions of the endocannabinoid. To achieve these goals, we used human cultured KCs (NHEKs, HaCaTs) and skin organ-culture models and employed combined pharmacological and molecular approaches.

RESULTS

Cultured human KCs express CB_1, CB_2, and TRPV1

First, we sought to identify the existence of putative AEA target molecules on human KCs that were shown to express ECS (Maccarrone *et al.*, 2003; Petrosino *et al.*, 2010). Expressions of CB_1 and CB_2 were unambiguously identified, both on NHEK and HaCaT KCs, using complementary immunocytochemistry and western blotting techniques (Figure 1a and b). Likewise, the "ionotropic AEA-receptor" TRPV1 was also detected on these cells (Figure 1a and b). This corresponded well to expression of the CB_1 and CB_2 genes in both types of KCs, as demonstrated by RT-PCR (Figure 1c) and by quantitative "real-time" PCR (Supplementary Figure 1 online; see also Supplementary Data online for the Materials and Methods).

AEA inhibits human KC growth and induces cell death

We then investigated the effects of the endocannabinoid on growth and survival of KCs. We found that AEA dose-dependently reduced ($P<0.05$, $n=4$ in each experiment) cell viability and proliferation of both NHEK and HaCaT cells (Figure 2a). To assess whether this effect was due to the induction of cell death (apoptosis and/or necrosis), a series of functional assays was performed. As measured by quantitative fluorimetric determinations (as well as by complementary immunofluorescence (data not shown)), AEA markedly ($P<0.05$) and dose-dependently increased the number of Annexin-V-positive cells (Figure 2b). Moreover, the endocannabinoid AEA markedly decreased mitochondrial membrane potential (Figure 2c) and induced the activation of pro-apoptotic caspases (Figure 2d), another hallmark of apoptosis. Of further importance, highest concentrations of AEA were also able to significantly ($P<0.05$) increase Sytox Green accumulation (Figure 2e) and glucose-6-phosphate dehydrogenase (G6PD) release (Figure 2f), two complementary indicators of necrosis/cytotoxicity. These findings suggested that AEA induced cell death of human KCs *in vitro*.

AEA inhibits proliferation and induces apoptosis of epidermal KCs *in situ*

Next, we employed the organ culture of normal, full-thickness human skin fragments (Lu *et al.*, 2007; Tiede *et al.*, 2009). After treatment with AEA, double Ki67/TUNEL immunolabeling was performed to simultaneously assess the *in situ* effects of AEA on human KC proliferation and apoptosis. As shown in Figure 3, perfectly in line with the above-mentioned cell culture results, AEA treatment markedly ($P<0.05$) suppressed the percentage of proliferating (Ki67+) cells, whereas it dramatically increased that of apoptotic (TUNEL+) cells in normal human epidermis organ cultured under serum-free conditions.

Cellular actions of AEA are mediated by CB_1 and TRPV1

We then investigated the involvement of "AEA-receptors" in mediating the cellular actions of the endocannabinoid. As AEA can stimulate both CBs and TRPV1, first, cultured human KCs were treated with highly selective inhibitors of defined CB subtypes (AM251 for CB_1 and AM630 for CB_2) or TRPV1 (capsazepine, iodoresiniferatoxin). Figure 4a shows that these inhibitors did not reduce viability of the cells. However, inhibition of CB_1 and TRPV1, but notably not of CB_2 alone, markedly abrogated the growth-inhibitory and apoptosis-inducing cellular effects of AEA ($P<0.05$; Figure 4b–d). Likewise, suppression of extracellular $[Ca^{2+}]$ also prevented the cellular actions of AEA (Figure 4b–d), further supporting the involvement of the Ca^{2+}-permeable ion channel TRPV1. (Suppression of extracellular $[Ca^{2+}]$ did not change the viability of the cells (data not shown).)

To further assess the roles of CB_1 and TRPV1, a series of receptor knockdown experiments was carried out in accordance with the techniques developed in our previous studies, which were optimized for various cultured skin cells (Dobrosi *et al.*, 2008; Tóth *et al.*, 2009). (The efficacy of the specific RNA interference (RNAi)-mediated silencing is shown in Supplementary Figure 2 online.) Scrambled RNAi probes or RNAi oligonucleotides against CB_1, CB_2, and TRPV1 did not decrease human KC viability in culture (Figure 5a). In contrast, RNAi-mediated silencing of CB_1 and TRPV1 moderately, yet significantly ($P<0.05$), stimulated KC growth (Figure 5a). This latter finding suggested that CB_1 and TRPV1 may function as constitutively active and/or continuously activated receptors (by endogenous ligands produced, for

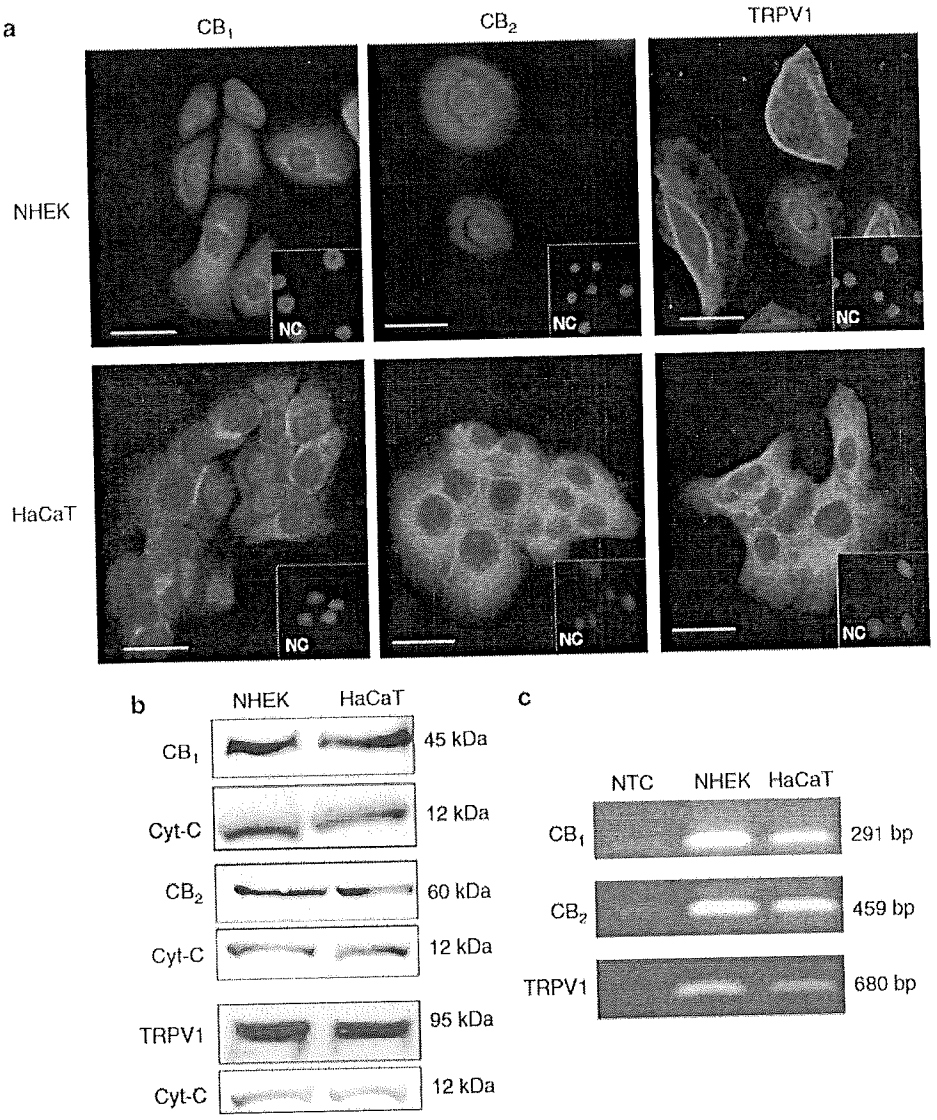

Figure 1. CB_1, CB_2, and transient receptor potential vanilloid-1 (TRPV1) are expressed on cultured human keratinocytes (KCs). (**a**) Specific immunoreactivity of CB_1, CB_2, and TRPV1 on normal human epidermal (NHEK; upper row) and HaCaT KCs (lower row), as determined by immunofluorescence (FITC, green fluorescence). Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue fluorescence). NC, pre-absorption negative control. Bar = 10 μm. (**b**) Western blot analysis. Expressions of CB_1, CB_2, and TRPV1 were determined on cell lysates of NHEK and HaCaT KCs. Cytochrome *c* (Cyt-C) served as loading control. (**c**) RT-PCR analysis of CB_1, CB_2, and TRPV1 mRNA transcripts. NTC, non-template control. In all cases, three to five additional experiments yielded similar results.

example, by KCs; Maccarrone *et al.*, 2003; Petrosino *et al.*, 2010) to inhibit the growth of epidermal KCs.

Similar to the above pharmacological data, silencing of CB_1 or TRPV1 (but, not of CB_2) counteracted AEA's negative effect on cell viability (Figure 5b) and the induction of apoptosis (Figure 5c and d). These data further argued in support of a simultaneous involvement of both CB_1- and TRPV1-mediated signaling.

A sequential signaling pathway ($CB_1 \rightarrow$ TRPV1 $\rightarrow$ Ca^{2+} influx) mediates the actions of AEA on human KCs

Intriguingly, the co-administration of CB_1 and TRPV1 antagonists (Figure 4b-d) or the simultaneous RNAi-mediated silencing of CB_1 and TRPV1 (Figure 5b d) did not exert additive effects in preventing the cellular actions of AEA. This suggested that AEA does not co-activate CB_1 and TRPV1, but rather induces sequential activation of either receptor (that is, $CB_1 \rightarrow$ TRPV1, or TRPV1 $\rightarrow CB_1$), which then mediates the complex actions of the endocannabinoid.

As TRPV1 functions as a Ca^{2+}-permeable ion channel on KCs as well (Southall *et al.*, 2003; Bodó *et al.*, 2005), Ca^{2+}-imaging experiments were performed to test whether AEA is capable of elevating the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). As a positive control, we employed the TRPV1 agonist capsaicin (CAPS), which increases $[Ca^{2+}]_i$ in KCs (Southall *et al.*, 2003; Bodó *et al.*, 2005).

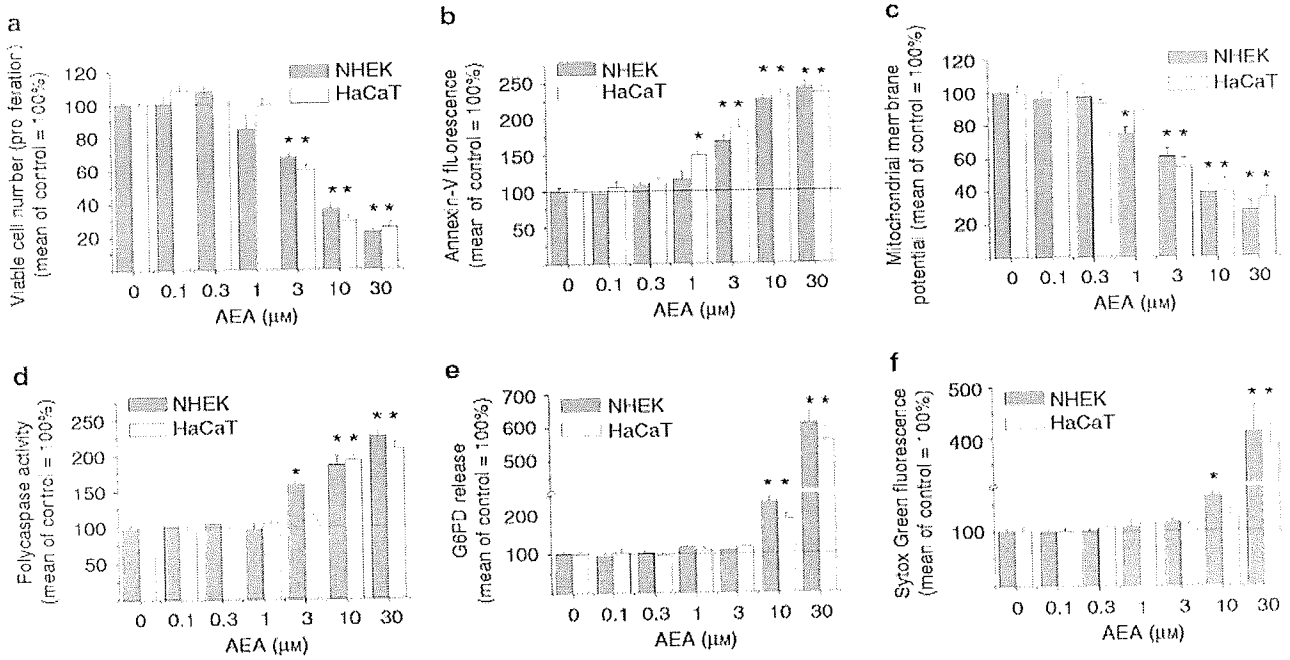

Figure 2. *N*-arachidonoylethanolamine (AEA) suppresses cellular viability and proliferation, and induces cell death of cultured human keratinocytes (KCs). KCs were treated with various concentrations of AEA for 24 hours. (**a**) Determination of viable cell number by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Quantitative measurement of apoptosis by (**b**) Annexin-V assay reflecting phosphatidylserine translocation; (**c**) $DiIC_1(5)$ assay reflecting mitochondrial membrane potential; (**d**) polycaspase assay reflecting activation of pro-apoptotic caspases. Quantitative measurement of necrosis by (**e**) glucose-6-phosphate dehydrogenase (G6PD) release assay and (**f**) Sytox Green assay. In all cases, data (mean ± SEM) are expressed as a percentage of the mean value (defined as 100%) of the vehicle-treated control group. For statistical analysis, * marks significant ($P < 0.05$) differences compared with the vehicle-treated control group; $n = 4$ in each group. Three to four additional experiments yielded similar results. NHEK, normal human epidermal KC.

AEA indeed induced transient elevations of $[Ca^{2+}]_i$ in a dose-dependent manner (Figure 6a). Of note, the amplitude of the maximal AEA-induced $[Ca^{2+}]_i$ elevation was in the range of that evoked by 10 μM CAPS (Figure 6a). However, the kinetics of the AEA- and the CAPS-induced cellular actions were markedly different. Namely, the effect of AEA was realized only after a long-term incubation of the cells (time-to-peak, TTP, value of 168.2 ± 20 seconds, mean ± SEM, $n = 16$ cells), in contrast to the fast action of the TRPV1 agonist CAPS (TTP of 13.8 ± 4 seconds, mean ± SEM, $n = 11$ cells; Figure 6b).

Of further importance, the AEA-evoked $[Ca^{2+}]_i$ transients were completely prevented by administering the TRPV1 antagonists capsazepine or iodoresiniferatoxin, or by suppression of extracellular $[Ca^{2+}]$ (Figure 6c). These findings further suggested that AEA induced a TRPV1-mediated Ca^{2+} influx. Likewise, the effect of AEA to raise $[Ca^{2+}]_i$ was also fully abrogated by the CB_1 antagonist AM-251 (Figure 6c). This argued for the fact that, besides TRPV1, CB_1-coupled signaling is also involved in mediating the effect of AEA to elevate $[Ca^{2+}]_i$. Finally, we have also found that co-administration of CB_1 and TRPV1 antagonists did not exert an additive inhibitory effect (Figure 6c).

Intriguingly, CB_1 and TRPV1 inhibitors behaved in a different way on the TTP value of the transients. Namely, the TRPV1 "channel antagonists" capsazepine and iodoresiniferatoxin did not affect the TTP value of AEA-induced $[Ca^{2+}]_i$ responses (Figure 6b). In striking contrast, the selective CB_1 antagonist AM-251 further markedly increased (the already long) TTP value by almost threefold (Figure 6b). (Instead, the CB_2 antagonist AM-630 had no effect on either the amplitude or the TTP of the AEA-evoked $[Ca^{2+}]_i$ elevations; Figure 6b and c.) Finally, as expected, we also found that the effect of CAPS to raise $[Ca^{2+}]_i$ was markedly abrogated by the TRPV1 inhibitors as well as by suppression of extracellular $[Ca^{2+}]$, whereas it was not affected by antagonists of CB_1 or CB_2 (Figure 6d).

DISCUSSION

Uncovering the as yet ill-characterized functions of the ECS in human skin biology and pathology is an important, integral part of the ongoing exploration of the skin as a non-classical neuroendocrine organ (Slominski and Wortsman, 2000; Slominski *et al.*, 2008). In this context, we provide evidence that the prototypic endocannabinoid AEA—which, as detailed above, is synthesized in several human skin cell compartments—inhibits proliferation and induces cell death of human epidermal KCs in cultures as well as *in situ*. We also show that AEA-induced KC death is Ca^{2+} dependent. These data support the concept that KCs exploit a physiologically relevant ECS for negatively regulating their own growth in a paracrine and/or autocrine manner.

Furthermore, we show that the KC death-promoting effects of AEA are mediated by a sequentially engaged signaling

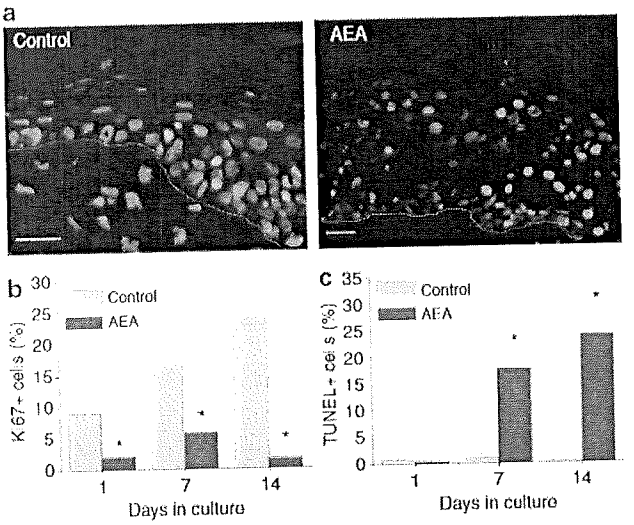

Figure 3. *N*-arachidonoylethanolamine (AEA) suppresses proliferation and induces apoptosis of human keratinocytes (KCs) *in situ*. Human skin organ cultures were treated for 1, 7, and 14 days by vehicle (Control) or 30 μM AEA. Cryostat sections were prepared and co-immunolabeling of proliferating (Ki67 +) and apoptotic (TUNEL +) cells was performed. (a) Representative immunofluorescence images of the epidermis after 14 days treatment. Ki67 + cells: red fluorescence, TUNEL + cells: green fluorescence. Nuclei were counterstained with DAPI (4′,6-diamidino-2-phenylindole; blue fluorescence). Dotted lines represent the border of epidermis and dermis. Bars = 25 μm. (b,c) Statistical analysis of number of Ki-67 + (b) and TUNEL + (c) cells, as compared with the number of DAPI + epidermal cells on 10 sections per group. Data are expressed as mean ± SEM. * Marks significant ($P < 0.05$) differences compared with the vehicle-treated control group. Three additional experiments yielded similar results.

mechanism ($CB_1 \rightarrow TRPV1 \rightarrow Ca^{2+}$ influx). This model is supported by several lines of evidence:

(i) Both CB_1 and TRPV1 antagonists, the suppression of extracellular $[Ca^{2+}]$, and RNAi-mediated silencing of these receptors were able to prevent the cellular actions of AEA.

(ii) However, these pharmacological and molecular inhibitory effects were not additive, arguing for the lack of co-activation of CB_1 and TRPV1 by AEA.

(iii) The effect of AEA to increase $[Ca^{2+}]_i$ was realized only after a long-term incubation of the cells, in contrast to the immediate action of the "direct" TRPV1 agonist CAPS. Although we cannot exclude the possibility that anandamide exhibited a slower onset of action due to its higher lipophilicity, as compared with that of CAPS (as nicely shown by Lazar *et al.*, 2006 and Ursu *et al.*, 2010), these results suggest that AEA may not directly activate TRPV1, but rather multiple (and yet to be determined) AEA-evoked signaling pathways are involved in the opening of the ion channel.

(iv) That these "upstream" mechanisms involve the preceding action of AEA on CB_1 is supported by the fact that, whereas both CB1 and TRPV1 antagonists were able to equally suppress the amplitude of the AEA-induced $[Ca^{2+}]_i$ elevations, the CB_1 antagonist AM-251 (unlike the "channel antagonists" of TRPV1 that do not affect the activity of CB_1) markedly increased the already very long TTP value of the $[Ca^{2+}]_i$ transients.

(v) Finally, we found that the effect of CAPS to raise $[Ca^{2+}]_i$ was inhibited by TRPV1 antagonists and by suppression of extracellular $[Ca^{2+}]$, but not by antagonists of CB_1, which argues for the lack of an "other way around" sequential mechanism of $AEA \rightarrow TRPV1 \rightarrow CB_1 \rightarrow Ca^{2+}$ influx.

This sequential activation mechanism, at least in part, is similar to those described in other cellular systems. Using cells ectopically co-expressing CB_1 and TRPV1, Hermann *et al.* (2003) have elegantly shown that CB_1 agonists, depending on the activity of the cAMP-coupled signaling mechanisms, may significantly modulate the Ca^{2+} influx mediated by TRPV1. Likewise, in experiments employing CB_1 gene-deficient mice, it was shown that constitutive activity at the CB_1 receptor was required to maintain the TRPV1 receptor in a "sensitized" state (Fioravanti *et al.*, 2008).

As inhibition or RNAi-mediated silencing of CB_2 did not affect the cellular actions of AEA (which, otherwise, may also activate CB_2; Pertwee, 2005; Mackie, 2006), it appears that CB_2 is not involved in mediating the growth-inhibitory effect of AEA on human epidermal KCs. These results were in line with previous findings showing that CB_1, but not CB_2, has a pivotal role in regulation of epidermal differentiation of human KCs (Paradisi *et al.*, 2008). However, CB_2-mediated signaling on KCs was shown to be involved in antinociception (by the release of endogenous opioids which, in turn, inhibit pain-sensing skin afferent fibers; Ibrahim *et al.*, 2005) and in various forms of cutaneous inflammation (Oka *et al.* 2006; Karsak *et al.*, 2007).

The fact that RNAi-mediated silencing of CB_1 and TRPV1 significantly promoted the growth of human KCs suggests that the joint CB_1-TRPV1 signaling pathway identified here functions as a previously unknown, endogenously active receptor-channel mechanism that constitutively keeps human KC proliferation in check. Therefore, the fine-tuned endogenous ECS tone of the skin—set by constant or on-demand production of locally synthesized endocannabinoids—not only controls, for example, cutaneous immune competence and/or tolerance, lipid homeostasis, or adnexal biology (reviewed in Bíró *et al.*, 2009; Kupczyk *et al.*, 2009) but also regulates epidermal homeostasis. Obviously, subsequent studies will need to support the physiological relevance of this, to our knowledge, previously unreported concept by direct *in vivo* evidence. Likewise, it deserves systematic analysis of whether dysfunctions in the cutaneous ECS can trigger or aggravate chronic hyperproliferative, pruritic, and/or pro-inflammatory skin diseases. Along these lines, the data reported here certainly encourage one to explore whether the targeted manipulation of the ECS could become a useful adjunct treatment strategy for hyperproliferative human dermatoses such as psoriasis or KC-derived skin tumors.

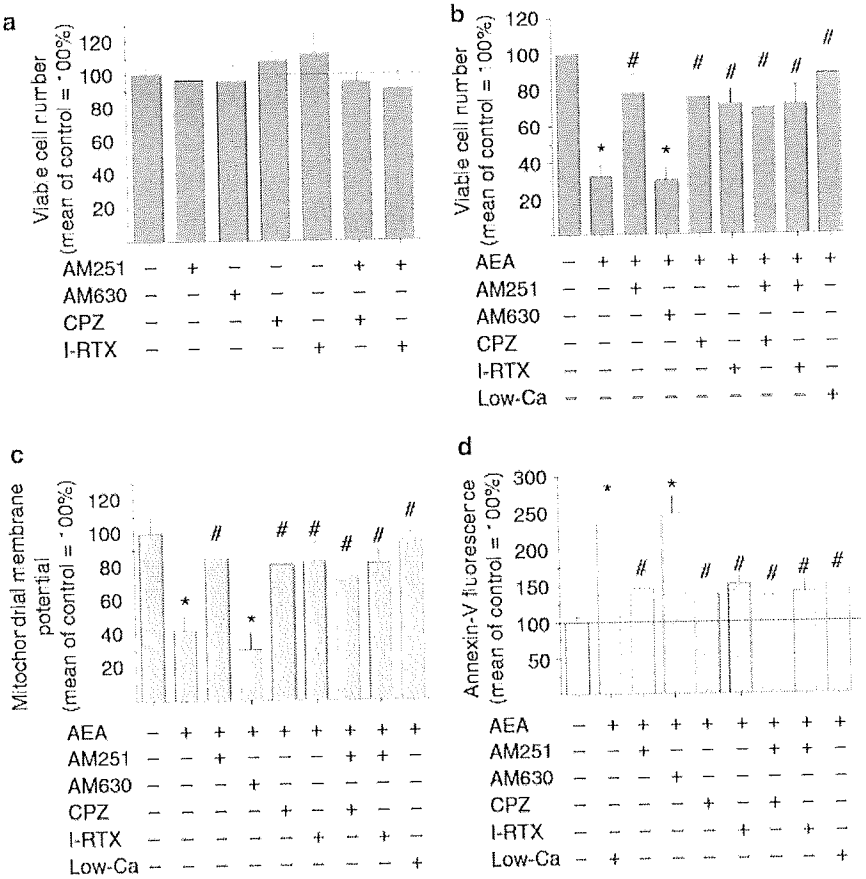

Figure 4. **Cellular effects of *N*-arachidonoylethanolamine (AEA) are inhibited by antagonists of CB_1 and transient receptor potential vanilloid-1 (TRPV1), and by the suppression of extracellular $[Ca^{2+}]$.** HaCaT keratinocytes (KCs) were treated for 24 hours by vehicle (control), 10 μM AEA, various antagonists: CB_1, 1 μM AM251; CB_2, 1 μM AM630; TRPV1, 5 μM capsazepine (CPZ); and 50 nM iodoresiniferatoxin (I-RTX). In addition, the effect of suppressing the $[Ca^{2+}]$ of the culturing medium from 2 to 0.02 mM (low-Ca) was also assessed. (**a**,**b**) Determination of viable cell number by MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay. Quantitative measurement of apoptosis by (**c**) $DiIC_1(5)$ assay and (**d**) Annexin-V assay. In all cases, data (mean ± SEM) are expressed as a percentage of the mean value (defined as 100%) of the vehicle-treated control group. For statistical analysis, * marks significant ($P < 0.05$) differences compared with the vehicle-treated control group, whereas # marks significant ($P < 0.05$) differences compared with the AEA-treated group; $n = 4$ in each group. Three to four additional experiments yielded similar results.

MATERIALS AND METHODS

Materials

AEA, AM-251, CAPS, capsazepine, and iodoresiniferatoxin were purchased from Sigma-Aldrich (St Louis, MO); AM630 was obtained from Tocris (Ellisville, MO).

Cell culturing

Human skin samples were obtained after obtaining written informed consent from healthy individuals undergoing dermatosurgery, adhering to Helsinki guidelines, and after obtaining Institutional Research Ethics Committee's permission. NHEKs were isolated after overnight dermo-epidermal separation in 2.4 U ml^{-1} dispase (Roche Diagnostics, Berlin, Germany) by short trypsin (0.05%, Sigma-Aldrich) digestion. Cells were cultured in EpiLife serum-free medium (Invitrogen, Paisley, UK), supplemented with 1 μM insulin, 1 μM cortisol (both from Sigma-Aldrich), 100 μg ml^{-1} streptomycin, 100 U ml^{-1} penicillin, 50 ng ml^{-1} amphotericin B (all from Biogal, Debrecen, Hungary), 0.4% bovine pituitary extract (Invitrogen), and 0.06 mM $CaCl_2$ (Sigma-Aldrich).

Human immortalized HaCaT KCs were cultured in DMEM (Sigma-Aldrich) supplemented with 10% fetal calf serum, 2 mM L-glutamine, and antibiotics (all from Invitrogen; Bodó *et al.*, 2005; Gönczi *et al.*, 2008; Kiss *et al.*, 2008; Szegedi *et al.*, 2009).

Experiments on full-thickness human skin organ cultures

Human skin fragments of standardized size and volume were generated and cultured in serum-free Williams' E medium (Biochrom, Cambridge, UK) supplemented with 2 mM L-glutamine, 10 ng ml^{-1} hydrocortisone (Sigma-Aldrich), 10 μg ml^{-1} insulin, and penicillin/streptomycin solution (PAA Laboratories, Pasching, Austria; Lu *et al.*, 2007; Tiede *et al.*, 2009).

To simultaneously assess proliferation and apoptosis in human skin organ cultures, a Ki67/TUNEL double-staining method was employed (Foitzik *et al.*, 2000; Lindner *et al.*, 2000; Bodó *et al.*, 2005). Briefly, after AEA treatment, cryostat sections were first labeled with a digoxigenin–dUTP (ApopTag Fluorescein *In Situ* Apoptosis Detection Kit, Millipore, Billerica, MA) in the presence of terminal deoxynucleotidyl transferase and then with a mouse

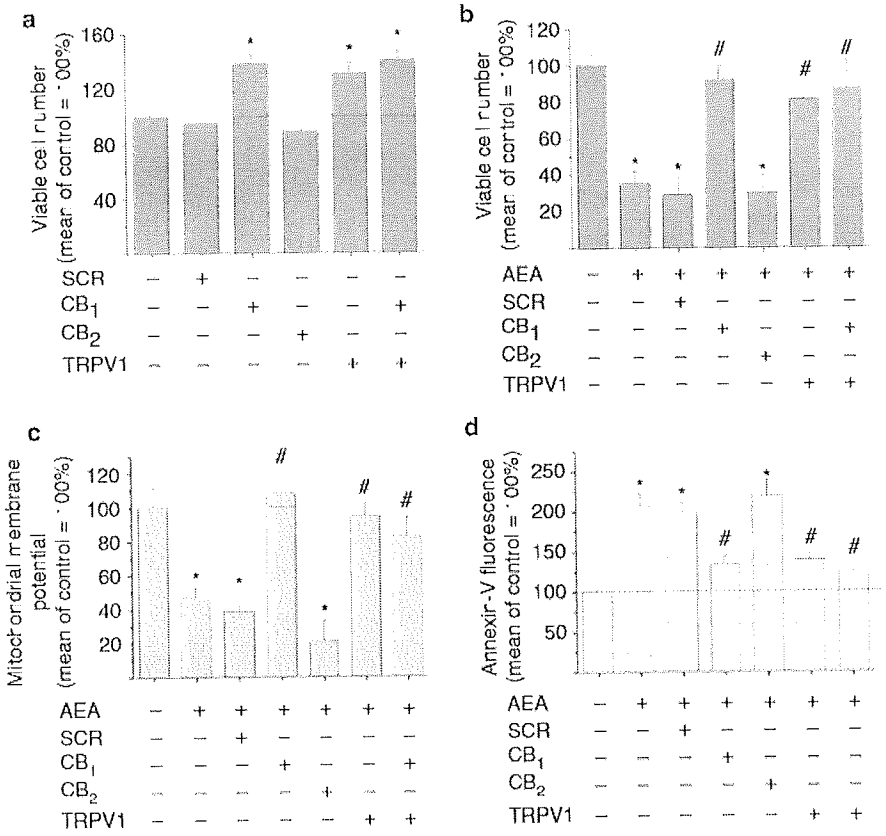

Figure 5. Cellular effects of *N*-arachidonoylethanolamine (AEA) are inhibited by RNA interference (RNAi)-mediated silencing of CB_1 and transient receptor potential vanilloid-1 (TRPV1), but not of CB_2. RNAi probes against CB_1, CB_2, and TRPV1, as well as scrambled RNAi probes (SCR), were introduced to HaCaT keratinocytes. Two days after transfection, cells were treated by 10 μM AEA for 24 hours. (**a**, **b**) Determination of viable cell number by colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Quantitative measurement of apoptosis by (**c**) $DiIC_1(5)$ assay and (**d**) Annexin-V assay. In all cases, data (mean ± SEM) are expressed as a percentage of the mean value (defined as 100%) of the vehicle-treated control group. For statistical analysis, * marks significant ($P < 0.05$) differences compared with the SCR group, whereas # marks significant ($P < 0.05$) differences compared with the AEA + SCR group; $n = 4$ in each group. Three additional experiments yielded similar results.

anti-Ki67 antiserum (1:20, DAKO, Hamburg, Germany). TUNEL + cells were visualized by an anti-digoxigenin FITC-conjugated antibody (ApopTag kit), whereas Ki67 + cells were labeled with a rhodamine-conjugated goat anti-mouse secondary antibody (1:200, Jackson ImmunoResearch, West Grove, PA). Sections were then counterstained with DAPI (4′,6-diamidino-2-phenylindole; Vector Laboratories, Burlingame, CA). Negative controls were performed by omitting terminal deoxynucleotidyl transferase and the Ki67 antibodies. The analysis for cell counting on 10 sections per group was performed using a fluorescence microscope BZ-8100 (Biozero, Keyence, Osaka, Japan). The distance between two analyzed sections was more than 50 μm.

Immunocytochemistry

NHEK and HaCaT KCs were incubated with rabbit primary antibodies against CB_1, CB_2 (1:200 dilution, Cayman, Ann Arbor, MI), and TRPV1 (1:100, Sigma-Aldrich). For fluorescence staining, slides were then incubated with FITC-conjugated secondary antibodies (dilution 1:200, Vector Laboratories) and the nuclei were visualized using DAPI. As negative controls, the appropriate antibody was either omitted from the procedure or was pre-incubated with synthetic blocking peptides used in two times excess concentrations for 1 hour. (Figure 1a, insets; Bodó *et al.*, 2005; Dobrosi *et al.*, 2008; Tóth *et al.*, 2009).

Western blotting

KCs were harvested and lysed in ice-cold lysis buffer (20 mM Tris-HCl (pH 7.5), 5 mM EGTA, protease inhibitor cocktail 1:100, all from Sigma-Aldrich). After determining the protein content, 100 μg protein from each sample was subjected to SDS-PAGE, transferred to BioBond nitrocellulose membranes (Whatman, Maidstone, UK), and then probed with the above-mentioned antibodies against CB_1, CB_2 (both in the ratio 1:200) and TRPV1 (1:100). A horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (1:1,000, Bio-Rad, Hercules, CA) was used as a secondary antibody, and the immunoreactive bands were visualized by a SuperSignal West Pico Chemiluminescent Substrate-Enhanced Chemiluminescence kit (Pierce, Rockford, IL) using LAS-3000 Intelligent Dark Box (Fuji, Tokyo, Japan). To assess equal loading, membranes were re-probed with an anti-cytochrome *c* antibody (1:50, Santa Cruz Biotechnology, Santa Cruz, CA) and visualized as described above.

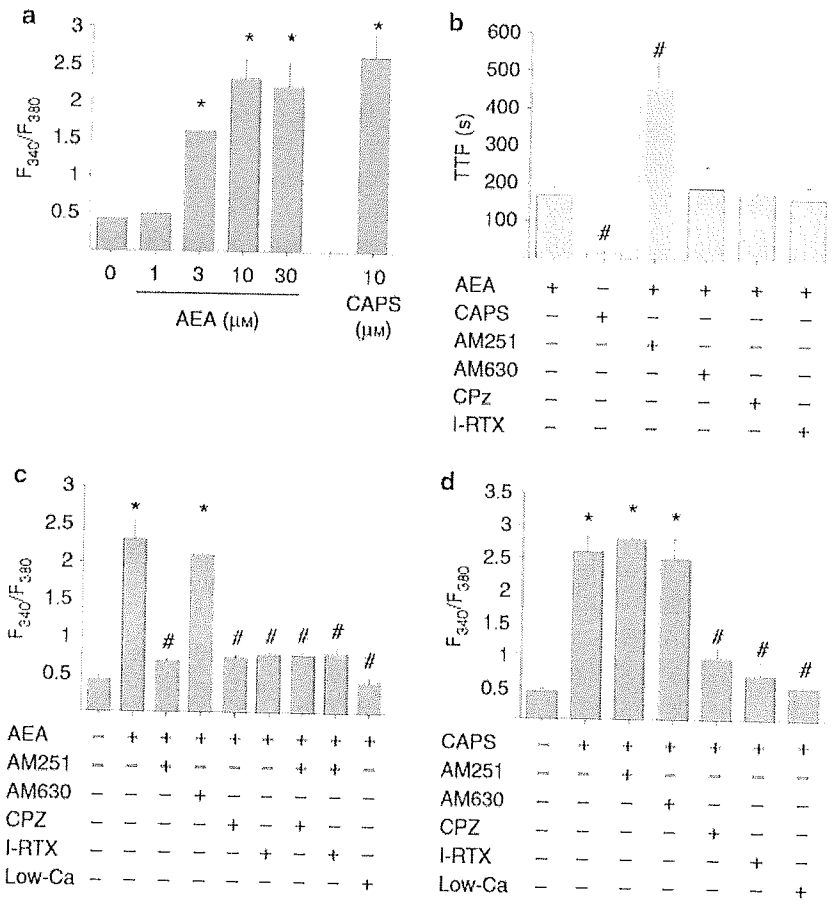

Figure 6. *N*-arachidonoylethanolamine (AEA) induces elevations of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) by antagonists of CB_1 and transient receptor potential vanilloid-1 (TRPV1), and by the suppression of extracellular $[Ca^{2+}]$. Ca^{2+} imaging on fura-2-loaded HaCaT keratinocytes. Fluorescence ratio (F_{340}/F_{380}) values of excitations at 340 and 380 nm wavelengths were recorded. (**a**) Effects of increasing doses on AEA and 10 μM capsaicin (CAPS). (**b**, **c**) Effects of various antagonists (CB_1: 1 μM AM251; CB_2: 1 μM AM630; TRPV1: 5 μM CPZ; and 50 nM I-RTX) as well as of suppressing the $[Ca^{2+}]$ of the medium from 2 to 0.02 mM (low-Ca) on the time-to-peak (TTP) values (**b**) and amplitudes (**c**) of Ca^{2+} transients evoked by 10 μM AEA. (**d**) Effects of antagonists and of suppressing extracellular $[Ca^{2+}]$ on the amplitudes of Ca^{2+} transients evoked by 10 μM CAPS. In all cases, mean ± SEM of multiple determinations ($n \geq 10$ cells) are presented. For statistical analysis, * marks significant ($P < 0.05$) differences compared with the vehicle-treated group, whereas # marks significant ($P < 0.05$) differences compared with the AEA-treated (**b**,**c**) or CAPS-treated (**d**) groups.

RT-PCR

Total RNA was isolated using TRIzol (Invitrogen), and the isolated total RNA was reverse-transcribed into cDNA and then amplified on a GeneAmp PCR System 2400 DNA Thermal Cycler (Applied Biosystems, Foster City, CA). Primers were synthesized by Invitrogen (CB_1, forward: 5′-CAAGCCCGCATGGACATTAGGTTA-3′, reverse: 5′-TCCGAGTCCCCCATGCTGTTATC-3′, 291 bp, accession number: NM_016083.4, non-intronspanning; CB_2, forward: 5′-TCCCACTGA TCCCCAATGACTACC-3′, reverse: 5′-AGGATCTCGGGGCTTCT TCTTTTG-3′, 459 bp, accession number: NM_001841.2, non-intronspanning; TRPV1 forward: 5′-CTCCTACAACAGCCTGTAC-3′, reverse: 5′-AAGGCCCAGTGTTGACAGTG-3′, 680 bp, accession number: NM_080704.3, intronspanning; glyceraldehyde-3-phosphate dehydrogenase, forward: 5′-ATGGTGAAGGTCGGTGTGAAC-3′, reverse: 5′-GCTGACAATCTTGAGGGAGT-3′, 340 bp, accession number: NM_002046.3, non-intronspanning). PCR products were visualized on 1.5% agarose gel with ethidium bromide (0.5 mg ml^{-1}, Sigma-Aldrich) under UV light.

Determination of viable cell numbers and proliferation

Cells were plated in 96-well multi-titer plates (20,000 cells per well density, which corresponded to approximately 70–80% confluence) in quadruplicates, and the number of viable cells (hence, the rate of proliferation) was determined by measuring the conversion of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide; Sigma-Aldrich) to formazan by mitochondrial dehydrogenases (Tóth *et al.*, 2009). In all cases, experiments were repeated at least three times.

Determination of apoptosis

A decrease in the mitochondrial membrane potential is one of the earliest markers of apoptosis (Green and Reed, 1998; Susin *et al.*, 1998). Mitochondrial membrane potential of cells was determined using a MitoProbe $DiIC_1(5)$ Assay Kit (Invitrogen). Cells (20,000 cells per well) were cultured in 96-well plates and treated in quadruplicates. After treatment, cells were incubated for 30 minutes with $DiIC_1(5)$ dye. Fluorescence was measured at 630 nm excitation and

670 nm emission wavelengths using a fluorescence image plate reader (FLIPR; Molecular Devices, San Francisco, CA).

In addition, apoptosis was also assessed by measuring phosphatidylserine translocation with FITC-conjugated Annexin-V (1:100, Sigma-Aldrich). Fluorescence was measured at 490 nm excitation and 520 nm emission wavelengths using FLIPR. As a complementary approach, immunocytochemistry was also performed using the same labeling method (data not shown).

Finally, apoptosis was also determined by fluorimetric measurement of activation of pro-apoptotic caspases using a fluorescent inhibitor of caspases (FLICA methodology, Poly Caspases Detection Kit, Invitrogen). The fluorescent FLICA reagent specifically and covalently interacts with the active centers of activated caspases via a caspase-specific recognition sequence. KCs were incubated with FLICA reagent and fluorescence was measured at 490 nm excitation and 530 nm emission wavelengths using FLIPR (Dobrosi *et al.*, 2008; Tóth *et al.*, 2009). In all apoptosis assays, experiments were repeated at least three times.

Determination of cytotoxicity (necrosis)

Cells (20,000 cells per well) were cultured in 96-well plates and treated in quadruplicates with endocannabinoids and antagonists for 24 hours. Necrotic cell death was determined by measuring G6PD release by an enzymatic process that leads to the reduction of resazurin into red-fluorescent resorufin (G6PD Release Assay Kit, Invitrogen). Fluorescence emission of resorufin was monitored by the FLIPR device at 545 excitation and 590 emission wavelengths.

As the activity of the G6PD released from necrotic cells decreases over 24 hours, the cytotoxic effects of long-term treatment protocols were determined by Sytox Green staining (Invitrogen). The dye is able to penetrate (and then bind to the nucleic acids) only into necrotic cells with ruptured plasma membranes. Fluorescence of intranuclear SYTOX Green was measured at 490 nm excitation and 520 nm emission wavelengths using a FLIPR (Dobrosi *et al.*, 2008; Tóth *et al.*, 2009). In both cytotoxicity assays, experiments were repeated at least three times.

RNA interference

RNAi was performed according to our optimized protocols (Dobrosi *et al.*, 2008; Tóth *et al.*, 2009). In brief, KCs at 50–70% confluence were transfected with specific stealth RNAi oligonucleotides (40 nM) against CB_1 (ID no. HSS102082), CB_2 (ID no. HSS102087), and TRPV1 (ID no. HSS111305) using Lipofectamine 2000 (all from Invitrogen). For controls, RNAi-negative control duplexes (scrambled RNAi) were employed. The efficacy of RNAi-driven "knockdown" was daily evaluated by quantitative PCR and western blotting for 4 days. At days 2 and 3, all specific RNAi oligonucleotides resulted in >70% silencing of the given molecule (see Supplementary Figure 2 online and Supplementary Data online).

Calcium measurement

Changes in $[Ca^{2+}]_i$ on TRPV1 activation were detected as described before (Bodó *et al.*, 2005; Tóth *et al.*, 2009). KCs, cultured on glass coverslips, were loaded with a calcium-sensitive probe fura-2 AM (5 μM, Invitrogen) and were then placed on the stage of an inverted fluorescence microscope (Diaphot, Nikon, Tokyo, Japan). Excitation was alternated between 340 and 380 nm using a dual-wavelength monochromator (Deltascan, Photon Technology International, New Brunswick, NJ). The emission was monitored at 510 nm with a photomultiplier at an acquisition rate of 10 Hz per ratio, and the fluorescence ratio (F_{340}/F_{380}) values were determined. Experiments were carried out on >10 cells in each experimental group.

Statistical analysis

Data were analyzed using one-way ANOVA with Bonferroni's and Dunnett's *post hoc* probes, and $P<0.05$ values were regarded as significant differences.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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#22

Section G & Section H } ATTACHMENT

The purpose of this document and all attached and or accompanying supporting documents is for the purpose of including Psoriasis and Psoriatic Arthritis to the List of Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.

Section H: Attachment – cannabinoids have been shown to be effective in reducing sensitivity to painful stimuli.

Article - Journal of Neurophysiology

Cannabinoid Modulation of Cutaneous Aδ Nociceptors During Inflammation

Article citing this article Cannabinoid Type-1 Receptor Reduces Pain and Neurotoxicity Produced by Chemotherapy J. Neurosci. May 16, 2012 32:(20) 7091-7101

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Abstract

Previous studies have demonstrated that locally administered cannabinoids attenuate allodynia and hyperalgesia through activation of peripheral cannabinoid receptors (CB_1 and CB_2). However, it is currently unknown if cannabinoids alter the response properties of nociceptors. In the present study, correlative behavioral and in vivo electrophysiological studies were conducted to determine if peripheral administration of the cannabinoid receptor agonists arachidonyl-2′-chloroethylamide (ACEA) or (R)-(+)-methanandamide (methAEA) could attenuate mechanical allodynia and hyperalgesia, and decrease mechanically evoked responses of Aδ nociceptors. Twenty-four hours after intraplantar injection of complete Freund's adjuvant (CFA), rats exhibited allodynia (decrease in paw withdrawal threshold) and hyperalgesia (increase in paw withdrawal frequency), which were attenuated by both ACEA and methAEA. The antinociceptive effects of these cannabinoids were blocked by co-administration with the CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) but not with the CB_2 receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone (AM630). ACEA and methAEA did not produce antinociception under control, non-inflamed conditions 24 h after intraplantar injection of saline. In parallel studies, recordings were made from cutaneous Aδ nociceptors from inflamed or control, non-inflamed skin. Both ACEA and methAEA decreased responses evoked by mechanical stimulation of Aδ nociceptors from inflamed skin but not from non-inflamed skin,

and this decrease was blocked by administration of the CB_1 receptor antagonist AM251. These results suggest that attenuation of mechanically evoked responses of Aδ nociceptors contributes to the behavioral antinociception produced by activation of peripheral CB_1 receptors during inflammation.

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INTRODUCTION

Several studies have demonstrated that locally administered cannabinoids produce antinociception in animal models of both acute and persistent pain through peripheral mechanisms (for reviews, see Hohmann 2002; Mbvundula et al. 2004; Walker and Huang 2002; Walker et al. 1999). Two receptors for cannabinoids have been isolated and cloned to date, cannabinoid 1 (CB_1) and cannabinoid 2 (CB_2), and both are G-protein-coupled receptors (Matsuda et al. 1990; Munro et al. 1993) that have been localized to various tissues. CB_1 receptors are most commonly expressed on neurons, and their activation can decrease neuronal excitability by decreasing calcium channel conductance and increasing potassium channel conductance (for review, see Howlett et al. 2004). CB_2 receptors are predominately expressed on leukocytes, and their activation can produce a variety of different immunological effects (for review, see Klein et al. 2003; Massi et al. 2006).

Locally administered cannabinoids have been shown to produce antinociception through activation of CB_1 and CB_2 receptors. CB_1 receptor-mediated antinociception has been attributed to activation of CB_1 receptors expressed by primary afferent nociceptive dorsal root ganglion neurons (Ahluwalia et al. 2000; Hohmann and Herkenham 1999) and their peripheral nerve terminals (Amaya et al. 2006; Ständer et al. 2005). Conditional knockdown of CB_1 receptors in Nav1.8-expressing nociceptive sensory neurons prevents locally administered cannabinoids from producing antinociception in models of neuropathic

and inflammatory pain (Agarwal et al. 2007). Consistent with these observations, activation of CB_1 receptors decreased high-voltage-activated calcium currents (Khasabova et al. 2002, 2004; Ross et al. 2001) and reduced capsaicin-evoked calcium transients (Millns et al. 2001; Sagar et al. 2005) in nociceptive dorsal root ganglion neurons in vitro. A recent study also showed that mechanically evoked responses of primary afferent neurons are decreased by CB_1 receptor activation in vivo; however, the types of afferent fibers affected are not known (Kelly and Donaldson 2008). The precise mechanisms underlying CB_2 receptor-mediated antinociception remain unclear but likely involve both indirect and direct actions on neuronal tissue (for review, see Guidon and Hohmann 2008).

Although behavioral studies have indicated that activation of peripheral CB_1 and CB_2 receptors produces antinociceptive effects, it is currently unknown if cannabinoids alter the response properties of nociceptors and which subtypes of functionally identified nociceptors are cannabinoid-sensitive. In the present study, parallel behavioral and electrophysiological experiments were conducted to determine how peripherally mediated antiallodynia and antihyperalgesia produced by cannabinoids relate to changes in Aδ nociceptor activity and through which cannabinoid receptor subtype these changes occur. In behavioral studies, we examined the effects of intraplantar administration of the cannabinoid receptor agonists arachidonyl-2′-chloroethylamide (ACEA) and (R)-(+)-methanandamide (methAEA) on mechanical allodynia and hyperalgesia in rats with complete Freund's adjuvant-induced inflammation of the hindpaw. In parallel electrophysiological studies, we investigated the effects of intraplantar administration of ACEA and methAEA on mechanically evoked responses of cutaneous Aδ nociceptors innervating inflamed skin. Similar behavioral and electrophysiological studies were also conducted to compare the effects of locally administered ACEA and methAEA in inflamed skin to any changes produced in control, non-inflamed skin.

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METHODS

Subjects

A total of 542 adult, male, Sprague–Dawley rats weighing 280–350 g were used in this study. Animals were obtained from Harlan (Indianapolis, IN), housed on a 12-h light/dark schedule, and allowed ad libitum access to food and water. Experiments were performed during the light cycle. All procedures were approved by the Animal Care Committee at the University of Minnesota, and experiments were conducted according to the guidelines established by the International Association for the Study of Pain.

Induction of inflammation

Rats were anesthetized with a mixture of isoflurane gas in air (2% for induction and maintenance; Phoenix Pharmaceuticals, St. Joseph, MO) and received a single intraplantar injection of complete Freund's adjuvant (CFA; (Sigma Chemical, St. Louis, MO) or sterile isotonic saline as a control (Baxter, Deerfield, IL). CFA (1 mg/ml) and saline were given in a volume of 50 μl using a 28-gauge needle. Electrophysiological and behavioral experiments were performed 24 h after injection of CFA or saline.

Drug preparation and administration

The cannabinoid receptor agonists were *N*-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (ACEA) and (R)-*N*-(2-hydroxy-1-methylethyl)-5Z,8Z,11Z,14Z-eicosatet raenamide (methAEA). The CB_1 receptor antagonist was *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM251) which exhibits ≈300-fold selectivity over CB2 receptors (Gatley et al. 1996). The CB_2 receptor antagonist was 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone

(AM630) which exhibits ≈160-fold selectivity over CB_1 receptors (Hosohata et al. 1997; Pertwee et al. 1995). All drugs were obtained from Tocris Bioscience (Ellisville, MO). ACEA was supplied predissolved in ethanol (5 mg/ml). methAEA was supplied predissolved in a water soluble emulsion (Tocrisolve; 5 mg/ml). AM251 was dissolved in anhydrous ethanol (25 μg/μl). AM630 was dissolved in a vehicle containing 5% Tween80 and 5% DMSO in isotonic saline (20 μg/μl). All drugs were administered via subcutaneous intraplantar injection in a volume of 50 μl for behavioral studies or 20 μl for electrophysiological studies.

Behavioral studies

PAW WITHDRAWAL THRESHOLDS.

Paw withdrawal thresholds to mechanical stimuli were used as a measure of mechanical allodynia. Withdrawal thresholds were determined using a series of eight calibrated Semmes-Weinstein von Frey monofilaments of logarithmic incremental stiffness (0.40–15 g; Stoelting, Wood Dale, IL). Animals were placed on an elevated wire-mesh platform under individual plastic cages and allowed to acclimate to the testing environment for 30 min prior to testing. The monofilaments were applied to the mid-plantar surface of the hindpaw for 1–2 s with an interstimulus interval of 5–6 s. The paw withdrawal threshold (g) was calculated according to the methods described by Chaplan et al. (1994). Baseline measures were determined for each animal for three consecutive days prior to injection of saline or CFA. Mechanical allodynia was defined as a decrease in the paw withdrawal threshold.

PAW WITHDRAWAL FREQUENCIES.

The frequency of paw withdrawal from a standard mechanical stimulus was used to measure mechanical hyperalgesia. The frequency of paw withdrawal evoked by mechanical stimulation was determined using a

26 g Semmes-Weinstein von Frey monofilament (Stoelting). Animals were placed on an elevated wire-mesh platform under individual plastic cages and allowed to acclimate to the testing environment for 30 min prior to testing. The filament was applied to the mid-plantar surface of the hindpaw 10 times for 1–2 s each with an interstimulus interval of 5–6 s, and the paw withdrawal frequency (%) was determined. Baseline measures were determined for each animal for three consecutive days prior to injection of saline or CFA. Mechanical hyperalgesia was defined as an increase in the paw withdrawal frequency.

Experimental design for behavioral studies

Separate groups of animals were used for studies of paw withdrawal thresholds or paw withdrawal frequencies. After three consecutive days of baseline testing, animals received an intraplantar injection of either CFA or saline into the left hindpaw. Twenty-four hours after injection, animals were randomly divided into groups of 8–10 rats each. Paw withdrawal thresholds or paw withdrawal frequencies were determined for both hindpaws before and 30, 60, and 120 min after cannabinoid or vehicle administration into the left hindpaw. In a separate group of rats, ACEA or methAEA was injected into the contralateral hindpaw and paw withdrawal thresholds or frequencies were determined in the inflamed (ipsilateral) hindpaw. The doses of either ACEA or methAEA given were 0.1, 1, or 10 μg. AM251 or AM630 was each given in doses of 30 μg and was co-injected with either ACEA (10 μg) or methAEA (10 μg). Each animal was used in only one experiment, and the experimenter was blinded to the identity of the drug administered.

Electrophysiological studies

SURGICAL PREPARATION.

Rats were initially anesthetized with ketamine (100 mg/kg im) and xylazine (45 mg/kg im). The trachea was cannulated, and a catheter was placed in the external jugular vein to provide supplemental anesthesia

with sodium pentobarbital (10 $mg \cdot kg^{-1} \cdot h^{-1}$). Core body temperature was maintained at 37°C using a feedback-controlled heating pad (Harvard Apparatus, Holliston, MA).

ELECTROPHYSIOLOGICAL RECORDING.

Recordings were made from cutaneous afferent fibers of the left tibial nerve using a teased-fiber approach. The tibial nerve was dissected from the surrounding tissue, and the overlaying skin was sewn to a metal ring to form a pool that was filled with warm mineral oil. The tibial nerve was placed onto a mirror platform for fine dissection with sharpened Dumont No. 5 forceps (Fine Science Tools, Foster City, CA). Teased fibers were placed onto a tungsten wire electrode, and action potentials were recorded extracellularly. Action potentials were amplified, audio monitored, displayed on an oscilloscope, and stored on a PC computer for data analysis. Only fibers with clearly discriminated single unitary action potentials (units) were studied. Responses of individual units were analyzed off-line using a customized data analysis program (LabVIEW, version 5.1; National Instruments, Austin, TX).

IDENTIFICATION OF UNITS.

Afferent units were found by mechanically stimulating the plantar surface of the hindpaw with the experimenter's finger or by stimulation with calibrated von Frey monofilaments. Once a single unit was identified, its mechanical receptive field (RF) was marked on the skin using a felt-tipped pen.

CONDUCTION VELOCITY.

The conduction velocity was determined by electrically stimulating the skin outside the unit's RF with pin electrodes to electrically activate the unit (200 μs pulse width at 0.5 Hz). The unit was stimulated 1.5× its electrical threshold and the conduction latency was measured from the time of the electrical stimulus artifact to the evoked unitary action potential. Conduction distance was determined by measuring the

distance from the unit's RF to the recording electrode. Conduction velocity (m/s) was calculated by dividing conduction distance by conduction latency.

FUNCTIONAL CLASSIFICATION OF NOCICEPTORS.

Units were classified functionally according to their responsiveness to mechanical and heat stimulation. Mechanical stimuli used to classify units included light brushing with the tip of a cotton swab, mildly pinching with a pair of forceps, and application of von Frey filaments. Mechanical response thresholds were determined using a series of calibrated von Frey monofilaments and defined as the weight (g) required to evoke at least one impulse when applied to a unit's RF for 1 s. Heat stimuli were delivered using a feedback-controlled Peltier device (Yale Electronics, New Haven, CT) with a contact area of 1 cm^2. A unit was considered heat-responsive if it responded with at least one impulse to a stimulus temperature of 51°C for 5 s. Units were classified as Aδ nociceptors if they had conduction velocities between 2.4 and 25.0 m/s and exhibited a slowly adapting response to noxious pinch but not to light touch (Leem et al. 1993).

Experimental design for electrophysiological studies

Once an Aδ nociceptor was characterized, baseline responses evoked by a 26 g von Frey monofilament were determined. This stimulus was above the mechanical response threshold for all Aδ nociceptors sampled in the present study and was the same filament used to determine mechanical hyperalgesia in our behavioral studies. The monofilament was secured in a manipulator and lowered onto the mechanical RF for 5 s. The monofilament was applied twice to the same location with an interstimulus interval of 180 s. The number of evoked impulses and the discharge rate (from the first to the last evoked impulse) were averaged over the two trials. For nociceptors that exhibited ongoing activity, the number of impulses that occurred 5 s prior to the stimulus was subtracted from the number of impulses

evoked during stimulation. To assess the variability between stimulus trials, the number of impulses elicited during the second stimulus trial was expressed as a percent of the number of impulses elicited during the first stimulus trial (Wenk et al. 2006).

After baseline responses were determined, cannabinoid or vehicle was injected into the unit's RF. The needle was inserted outside the RF, and the injectate was observed as a bleb of fluid centered in the unit's RF. Ongoing activity was recorded before, during, and for 300 s after injection of drug or vehicle. Responses were separated into injection responses, the response during injection, and post-injection responses, the response after injection of drug and withdrawal of the needle from the skin. Injection and post-injection response magnitudes are indicated as both the number of impulses elicited and discharge rate (Hz). Mechanical response thresholds, mechanically evoked responses, and the variability between stimulus trials were determined 30, 60, 90, and 120 min after injection of drug or vehicle as described above. The dose of either ACEA or methAEA given was 10 μg. AM251 or AM630 was each given in doses of 30 μg and pre-injected prior to injection of ACEA (10 μg) or methAEA (10 μg). Only one nociceptor was studied per animal.

Data analysis

BEHAVIORAL STUDIES.

To determine the effect of cannabinoids or their vehicles on paw withdrawal thresholds and paw withdrawal frequencies compared with baseline measures, comparisons were made using one-way repeated-measures ANOVA followed by paired *t*-test with the Bonferroni correction for multiple comparisons. A one-way ANOVA followed by unpaired *t*-test with the Bonferroni correction for multiple comparisons was used to determine the effect of cannabinoids or their vehicles on paw withdrawal thresholds and paw withdrawal frequencies between groups. Within group comparisons of the main effect of CFA and saline

injection on paw withdrawal thresholds and paw withdrawal frequencies were made using paired *t*-tests. For all statistical analyses, a probability value <0.05 was considered significant. All data are presented as means ± SE.

ELECTROPHYSIOLOGICAL STUDIES.

To determine the effect of cannabinoids or their vehicles on mechanically evoked responses and variability between stimulus trials compared with baseline measures, comparisons were made using a one-way repeated-measures ANOVA followed by paired *t*-test with the Bonferroni correction for multiple comparisons. A two-way ANOVA followed by unpaired *t*-test with the Bonferroni correction for multiple comparisons was used to determine the effect of cannabinoids or their vehicles on mechanically evoked responses between groups. Between-group comparisons of conduction velocity and mechanically evoked responses on nociceptors isolated from CFA- and saline-injected hindpaws were made using unpaired *t*-tests. To determine the effect of cannabinoids or their vehicles on mechanical response thresholds compared with baseline measures, comparisons were made using the Kruskal-Wallis ANOVA followed by Mann-Whitney rank sum tests. To compare the effect of drug or vehicle on mechanical response thresholds between groups, Mann-Whitney rank sum tests were used. Injection and post-injection response magnitudes were compared between groups using one-way ANOVA followed by unpaired *t*-tests with the Bonferroni correction for multiple comparisons. The proportions of units exhibiting injection and post-injection responses between groups were made using χ^2 test followed by pair-wise comparisons using the Fisher exact test. For all statistical analyses, a probability value <0.05 was considered significant. All data are presented as means ± SE. All statistical analysis was performed using Sigma Stat software (Systat Software, San Jose, CA).

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RESULTS

Behavioral studies

MECHANICAL HYPERALGESIA AND MECHANICAL ALLODYNIA AFTER INDUCTION OF INFLAMMATION.

Twenty-four hours after intraplantar injection of CFA, rats exhibited mechanical allodynia and mechanical hyperalgesia in the injected hindpaw. Paw withdrawal thresholds decreased from 13.6 ± 0.2 to 5.0 ± 0.2 g (n = 140, $P < 0.001$), and paw withdrawal frequencies increased from 26.9 ± 0.7 to 94.8 ± 0.6% (n = 188, $P < 0.0001$). Paw withdrawal thresholds (n = 37) and frequencies (n = 32) did not change 24 h after intraplantar injection of saline (from 13.1 ± 0.3 to 12.9 ± 0.4 g and from 24.6 ± 1.7 to 25.7 ± 1.3%, respectively). No changes in withdrawal thresholds or withdrawal frequencies were observed in the contralateral hindpaw in either CFA- or saline-treated rats.

EFFECTS OF ACEA AND METHAEA ON MECHANICAL ALLODYNIA.

Intraplantar injection of ACEA or methAEA, but not vehicle, dose-dependently attenuated mechanical allodynia produced by CFA. Increases in withdrawal threshold occurred after the 1 and 10 μg doses of both cannabinoids (Fig. 1, *A* and *B*). The antiallodynic effects of both cannabinoids peaked 30 min after administration and withdrawal thresholds returned to baseline values by 60 min after administration (Fig. 1, *C* and *D*).

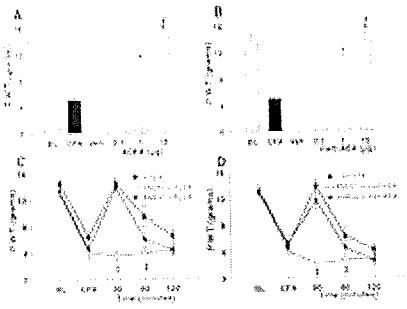

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FIG. 1.

arachidonyl-2′-chloroethylamide (ACEA) and (R)-(+)-methanandamide (methAEA) attenuate mechanical allodynia via activation of peripheral cannabinoid 1 (CB_1) receptors. Peripheral administration of either ACEA (*A*) or methAEA (*B*) dose-dependently increased paw withdrawal thresholds at doses of 1 and 10 μg (values shown indicate 30 min after administration). Co-administration with the CB_1 receptor antagonist *N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophen yl)-4-methyl-1H-pyrazole-3-carboxamide (AM251, 30 μg), but not the cannabinoid 2 (CB_2) receptor antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-y l](4-methoxyphenyl)methanone (AM630, 30 μg), blocked the antiallodynic effects of 10 μg ACEA (*C*) and 10 μg methAEA (*D*). BL, mean baseline paw withdrawal thresholds 24 h prior to injection of complete Freund's adjuvant (CFA); CFA, mean paw withdrawal thresholds 24 h after intraplantar injection of CFA and also serves as the predrug baseline; PWT, paw withdrawal threshold; Time, time after intraplantar injection of drugs; *, significant difference from vehicle ($P < 0.05$); #, significant difference from 1 μg of methAEA/ACEA ($P < 0.05$); ‡, significant difference from 10 μg of methAEA/ACEA ($P < 0.05$). $n = 8–10$ animals per dose.

To determine if the antiallodynia produced by ACEA and methAEA was mediated by peripheral cannabinoid receptors, rather than through a systemic mechanism, ACEA (10 μg) or methAEA (10 μg) was injected into the contralateral hindpaw and paw withdrawal thresholds were determined in the inflamed (ipsilateral) hindpaw. Injection of either ACEA or methAEA into the contralateral hindpaw did not alter withdrawal thresholds in the inflamed (ipsilateral) hindpaw (data not

shown). These results indicate that the antiallodynia after administration of ACEA and methAEA was mediated by peripheral cannabinoid receptors.

ACEA and methAEA were co-administered with either the CB_1 receptor antagonist, AM251, or the CB_2 receptor antagonist, AM630, to determine which cannabinoid receptor subtype mediated the antiallodynic effects produced by both cannabinoids. Co-administration of either ACEA (10 μg) or methAEA (10 μg) with AM251 (30 μg), but not AM630 (30 μg), blocked the increase in withdrawal thresholds produced by ACEA and methAEA (Fig. 1, *C* and *D*). These results suggest that the antiallodynia after administration of ACEA and methAEA are mediated by peripheral CB_1 receptors. Administration of AM251 or AM630 alone, or their vehicles, did not alter withdrawal thresholds (data not shown).

EFFECTS OF ACEA AND METHAEA ON MECHANICAL HYPERALGESIA.

Intraplantar injection of ACEA or methAEA, but not vehicle, dose-dependently attenuated mechanical hyperalgesia. Decreases in withdrawal frequencies occurred after the 1 and 10 μg doses for both cannabinoids (Fig. 2, *A* and *B*). The antihyperalgesic effects of both cannabinoids peaked 30 min after administration and paw withdrawal frequencies returned to baseline values by 60 min after administration (Fig. 2, *C* and *D*).

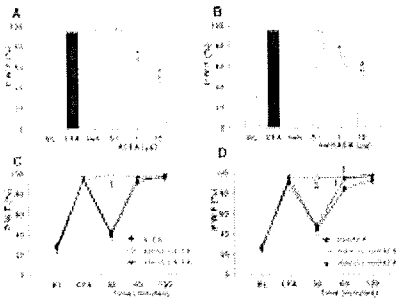

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FIG. 2.

ACEA and methAEA attenuate mechanical hyperalgesia by activation of peripheral CB_1 receptors. Peripheral administration of either ACEA (*A*) or methAEA (*B*) dose-dependently decreased paw withdrawal frequencies at doses of 1 and 10 μg (values shown indicate 30 min after administration). Co-administration with the CB_1 receptor antagonist AM251 (30 μg), but not the CB_2 receptor antagonist AM630 (30 μg), blocked the antihyperalgesic effects of 10 μg ACEA (*C*) and 10 μg methAEA (*D*). BL, mean baseline paw withdrawal frequency 24 h prior to injection of CFA; CFA, mean paw withdrawal frequency 24 h after intraplantar injection of CFA and also serves as the predrug baseline; PWF, paw withdrawal frequency; Time, time after intraplantar injection of drugs; *, significant difference from vehicle ($P < 0.05$); #, significant difference from 1 μg of methAEA/ACEA ($P < 0.05$); ‡, significant difference from 10 μg of methAEA/ACEA ($P < 0.05$). $n = 8–10$ animals per dose.

Similar to the results for mechanical allodynia, intraplantar injection of either ACEA (10 μg) or methAEA (10 μg) into the contralateral hindpaw did not alter paw withdrawal frequencies in the inflamed (ipsilateral) hindpaw (data not shown). Again, these results indicate that the antihyperalgesic effects of locally administered ACEA and methAEA are mediated by peripheral cannabinoid receptors.

The decrease in withdrawal frequencies produced by either ACEA (10 μg) or methAEA (10 μg) was blocked by the CB_1 receptor antagonist AM251 (30 μg) but not by CB_2 receptor antagonist AM630 (30 μg; Fig. 2, *C* and *D*). These results suggest that the antihyperalgesia produced by ACEA and methAEA is mediated by peripheral CB_1 receptors.

Administration of AM251 or AM630 alone, or their vehicles, did not alter paw withdrawal frequencies (data not shown).

EFFECTS OF ACEA AND METHAEA IN CONTROL, NON-INFLAMED RATS.

Twenty-four hours after intraplantar injection of saline, rats received an intraplantar injection of ACEA (10 μg), methAEA (10 μg), or vehicle. In contrast to the antinociceptive effects observed in CFA-injected rats, ACEA and methAEA, as well as their vehicles, produced a small decrease in paw withdrawal thresholds and a trend for an increase paw withdrawal frequencies (Fig. 3, *A–D*). The decrease in withdrawal thresholds produced by ACEA and methAEA did not differ from their vehicles at any time point tested. These results suggest that peripheral administration of ACEA or methAEA does not produce antinociception to mechanical stimuli during control, non-inflamed conditions.

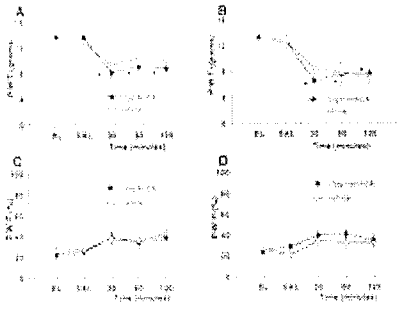

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FIG. 3.

Effects of ACEA and methAEA on withdrawal responses during non-inflamed conditions. Effects of intraplantar injection of ACEA (10 μg) or vehicle on mean paw withdrawal thresholds (*A*) and mean paw withdrawal frequencies (*C*) 24 h after intraplantar injection of saline.

Effects of intraplantar injection of methAEA (10 μg) or vehicle on mean paw withdrawal thresholds (*B*) and mean paw withdrawal frequencies (*D*) 24 h after intraplantar injection of saline. BL, mean baseline paw withdrawal threshold and withdrawal frequency 24 h before intraplantar injection of saline; SAL, mean paw withdrawal threshold and mean withdrawal frequency 24 h after intraplantar injection of saline and also serves as the predrug baseline; *, significant difference from SAL ($P < 0.05$). $n = 8–10$ animals per dose.

Electrophysiological studies

GENERAL PROPERTIES OF Aδ NOCICEPTORS.

A total of 145 Aδ nociceptors were studied: 40 from control, non-inflamed (saline-injected) skin and 105 from inflamed (CFA-injected) skin. The mean conduction velocity of Aδ nociceptors isolated from non-inflamed skin was 15.7 ± 0.6 m/s with a range of 4.2–20.8 m/s and was similar to the mean conduction velocity of Aδ nociceptors from inflamed skin (15.1 ± 0.4 m/s with a range of 3.1–21.8 m/s). Examples of conduction latency traces are displayed in Fig. 4*A*. The median mechanical response threshold of Aδ nociceptors from inflamed skin was 2.5 g (interquartile range = 3.4 g), which was lower than the median mechanical response threshold of Aδ nociceptors from non-inflamed skin (4.7 g; interquartile range = 4.3 g; $P < 0.001$). None of the Aδ nociceptors from non-inflamed skin exhibited ongoing activity, whereas 25% (26/105) of Aδ nociceptors from inflamed skin exhibited ongoing activity with an average discharge rate of 0.16 ± 0.03 Hz (range = 0.02–0.61 Hz). None of the Aδ nociceptors from non-inflamed skin were excited by noxious heat, whereas 4% (4/105) of Aδ nociceptors from inflamed skin were excited by heat. Examples of heat responses of a single Aδ nociceptor from inflamed skin are shown in Fig. 4*C*.

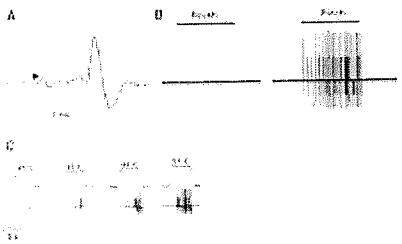

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FIG. 4.

Examples of Aδ nociceptor activity. *A*: 3 overlaying conduction latency traces of a single Aδ nociceptor from non-inflamed skin. Arrowhead indicates electrical stimulus artifact. *B*: the response of this nociceptor to noxious pinch but not brushing in its receptive field (RF). The line above each trace in *B* represents 2 s. *C*: responses of a single Aδ nociceptor from inflamed skin to increasing heat stimuli applied to the unit's RF.

MECHANICALLY EVOKED RESPONSES OF Aδ NOCICEPTORS.

Preliminary studies were initially conducted to determine the responses of Aδ nociceptors to graded mechanical stimuli. Examples of graded responses to mechanical stimulation are displayed in Fig. 5*A*. As expected, Aδ nociceptors from non-inflamed skin responded montonically to graded von Frey filaments of 10, 26, and 60 g (n = 6 per group; Fig. 5*B*). Similarly, Aδ nociceptors from inflamed skin also responded monotonically, although the magnitude of response to each stimulus was greater than those of Aδ nociceptors from non-inflamed skin (n = 6 per group; $P < 0.001$; Fig. 5*B*).

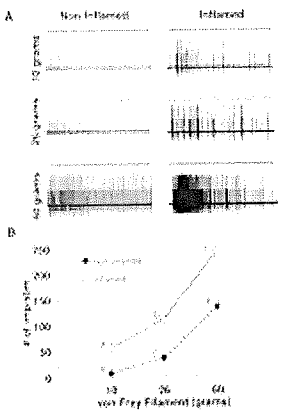

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FIG. 5.

Responses of Aδ nociceptors to mechanical stimulation. *A*: examples of responses of Aδ nociceptors evoked by stimulation with 10, 26, and 60 g von Frey monofilaments from non-inflamed (*left*) and inflamed (*right*) skin (line above each traces represent stimulation for 5 s). *B*: the mean number impulses evoked by stimulation with 10, 26, and 60 g von Frey filaments of Aδ nociceptors are shown. # of imp/stim: number of impulses elicited by stimulation with a von Frey monofilament for 5 s. Groups that do not share letters are significantly different ($P < 0.05$). $n = 6$ units per group.

We determined the effects of cannabinoids or vehicle on responses evoked by the 26 g von Frey filament, the same stimulus used in behavioral studies. A concern was the potential variability of responses to repeated application of the stimulus because responses at each time point represented are the average of two stimulus trials (see methods). We accounted for the variability of responses by expressing the number of impulses evoked during the second stimulus trial as a percent of the

number of impulses evoked during the first stimulus trial. Overall the variability between stimulus trials for baseline responses of Aδ nociceptors isolated from non-inflamed and inflamed hindpaws did not differ and was 110.8 ± 4.9% (n = 40) and 105.6 ± 3.0% (n = 105), respectively. Units were classified as cannabinoid-sensitive if the evoked responses after cannabinoid administration were 2 SD below the baseline response (Wenk et al. 2006). Thus units that had a decrease in response of ≤44% after cannabinoid administration were considered cannabinoid-sensitive.

Across all 40 Aδ nociceptors from non-inflamed skin, the mean baseline response to 26 g von Frey filament was 33.4 ± 1.9 impulses (6.6 ± 0.3 Hz). For Aδ nociceptors from inflamed skin (n = 105), the mean baseline response was 78.4 ± 3.9 impulses (16.0 ± 0.8 Hz) and was significantly greater than the responses of nociceptors from non-inflamed skin (P < 0.001).

EFFECTS OF METHAEA ON Aδ NOCICEPTORS FROM INFLAMED SKIN.

Administration of the cannabinoid receptor agonist methAEA (10 μg) attenuated mechanically evoked responses from 81.1 ± 10.1 to 42.3 ± 6.6 impulses (a decrease of ≈ 48%) that peaked 30 min after administration and returned to baseline values 60 min after administration (Fig. 6). Unlike the decreases in mechanically-evoked responses observed after injection of methAEA (10 μg), injection of vehicle increased mechanically evoked responses at all time points tested (P < 0.01) (Fig. 6). Seven of the 20 (35%) Aδ nociceptors treated with methAEA were cannabinoid-sensitive. Neither vehicle nor methAEA altered mechanical response thresholds or the variability in responses (number of evoked impulses) between stimulus trials at any time point tested (data not shown).

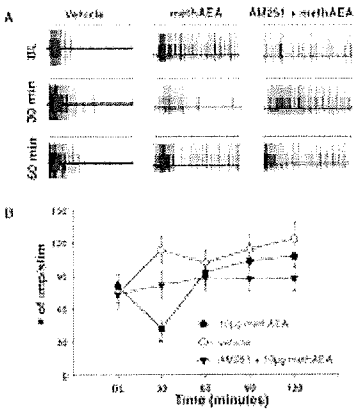

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FIG. 6.

Local administration of methAEA decreases mechanically evoked responses of Aδ nociceptors from inflamed skin by activation of CB_1 receptors. *A*: examples of mechanically-evoked responses via stimulation with a 26 g von Frey filament before and after administration of methAEA (10 μg), vehicle, or AM251 (30 μg) followed by methAEA (10 μg) are shown. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by stimulation with a 26 g von Frey filament before and after administration of methAEA (10 μg), vehicle, or AM251 (30 μg) followed by methAEA (10 μg) are shown. BL, mean baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from vehicle ($P < 0.05$). n = 15–20 units per group.

To determine if the attenuation of mechanically evoked responses by methAEA was mediated by CB_1 receptors, the CB_1 receptor antagonist AM251 (30 µg) was administered 5 min prior to injection of methAEA (10 µg). Pre-treatment with AM251 blocked the attenuation of mechanically evoked responses produced by methAEA (Fig. 6). No changes in mechanical response thresholds or the variability in responses between stimulus trials occurred at any time point tested after administration of AM251 followed by methAEA (data not shown).

EFFECTS OF ACEA ON Aδ NOCICEPTORS FROM INFLAMED SKIN.

Administration of the cannabinoid receptor agonist ACEA (10 µg) also attenuated mechanically evoked responses from 80.0 ± 9.1 to 51.3 ± 6.1 impulses (a decrease of ≈36%) that peaked at 30 min after administration and returned to baseline values 60 min after administration (Fig. 7). Unlike the decreases in mechanically-evoked responses observed after injection of ACEA, the injection of vehicle did not alter mechanically evoked responses. Six of the 17 (35%) Aδ nociceptors treated with ACEA were cannabinoid-sensitive. Neither vehicle nor ACEA altered mechanical response thresholds or the variability in responses between stimulus trials at any time point tested (data not shown).

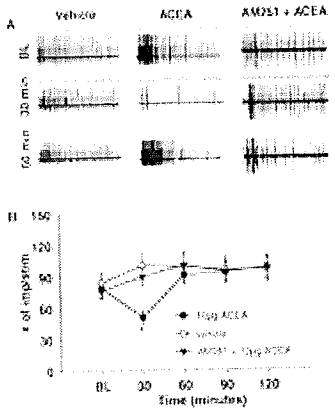

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FIG. 7.

Local administration of ACEA decreases mechanically evoked responses of Aδ nociceptors from inflamed skin by activation of CB_1 receptors. *A*: examples of mechanically evoked responses via stimulation with a 26 g von Frey filament before and after administration of ACEA (10 μg), vehicle, or AM251 (30 μg) followed by ACEA (10 μg) are shown. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by stimulation with a 26 g von Frey filament before and after administration of ACEA (10 μg), vehicle, or AM251 (30 μg) followed by ACEA (10 μg) are shown. BL, mean baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from vehicle ($P < 0.05$). n = 15–20 units per group.

As with methAEA, AM251 (30 μg) also blocked the attenuation of mechanically evoked responses produced by ACEA (Fig. 7). No changes in mechanical response thresholds or the variability in responses between stimulus trials occurred at any time point tested after administration of AM251 followed by ACEA (data not shown).

EFFECTS OF ACEA AND METHAEA ON Aδ NOCICEPTORS FROM NON-INFLAMED SKIN.

In contrast to the effects observed on Aδ nociceptors from inflamed skin, responses to the 26 g monofilament increased after methAEA (10 μg) or its vehicle (Fig. 8). The small increase in mechanically evoked responses produced by methAEA and vehicle did not differ at any time point tested. Neither methAEA nor its vehicle altered mechanical

response thresholds or variability in responses between stimulus trials at any time point tested. Similarly, administration of ACEA (10 μg) increased, and its vehicle did not alter, mechanically evoked responses compared with baseline measures (Fig. 9). As with methAEA, the changes in mechanically evoked responses produced by ACEA and vehicle did not differ at any time point tested. Neither ACEA nor vehicle altered mechanical response thresholds or the variability in responses between stimulus trials at any time point tested (data not shown).

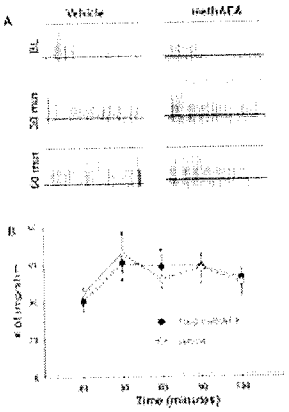

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FIG. 8.

Effect of methAEA on mechanically evoked responses of Aδ nociceptors from non-inflamed skin. *A*: examples of responses evoked by stimulation with the 26 g monofilament before and after administration of methAEA (10 μg) or vehicle. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by the monofilament before and after administration of methAEA (10 μg) or

vehicle are shown. BL, baseline predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from BL ($P < 0.05$). $n = 10$ units per group.

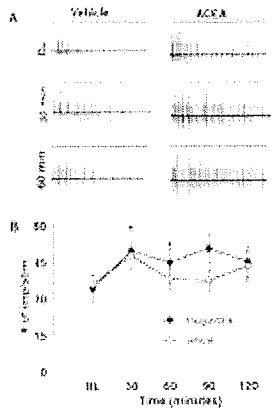

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FIG. 9.

Effect of ACEA on mechanically evoked responses of Aδ nociceptors from non-inflamed skin. *A*: examples of responses evoked by stimulation with the 26 g monofilament before and after administration of ACEA (10 μg) or vehicle. Each column represents responses of different Aδ nociceptors, and each row represents a different time point indicated on the *left*. The line above each trace represents the time of stimulation (5 s). *B*: the mean number impulses evoked by the monofilament before and after administration of ACEA (10 μg) or vehicle are shown. BL, predrug number of impulses; Time, time after administration of drug; # of imp/stim, number of impulses elicited by stimulation with a von Frey monofilament for 5 s; *, significant difference from BL ($P < 0.05$). $n = 10$ units per group.

RESPONSES EVOKED BY METHAEA, ACEA, AND THEIR VEHICLES.

Previous studies have demonstrated that both ACEA (Price et al. 2004) and methAEA (Ralevic et al. 2001; Roberts et al. 2002) are able to activate TRPV1 receptors. To account for this potential excitatory effect, we recorded nociceptor activity during injection of drug (injection response) and for a 5-min period after injection (post-injection response). Injection responses of Aδ nociceptors from non-inflamed skin did not differ in either proportion or magnitude regardless of the cannabinoid or vehicle injected. Similarly injection responses of Aδ nociceptors from inflamed skin did not differ in either proportion or magnitude regardless of the cannabinoid or vehicle injected. The magnitude of injection responses of Aδ nociceptors from inflamed skin, but not proportions, were greater than injection responses of Aδ nociceptors from non-inflamed skin ($P < 0.01$). Because only a small proportion of Aδ nociceptors from non-inflamed skin exhibited post-injection responses (5/40), differences between cannabinoids and vehicle could not be ascertained. Post-injection responses of Aδ nociceptors (53/105) from inflamed skin did not differ in either proportion or magnitude regardless of cannabinoid or vehicle injected. Overall the magnitude ($P < 0.01$) and proportion ($P < 0.0001$) of post-injection responses of Aδ nociceptors from inflamed skin were greater than those of Aδ nociceptors from non-inflamed skin. A complete listing of responses during and after injection is provided in Supplementary Table 1.[1] These data show that injection of cannabinoids or their vehicles into the plantar surface of the hindpaw evokes non-specific excitation of Aδ nociceptors, and inflammation increases the magnitude of this response.

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DISCUSSION

In the present study, local administration of the cannabinoid receptor agonists, methAEA or ACEA, attenuated inflammatory mechanical

allodynia and hyperalgesia and decreased mechanically evoked responses of Aδ nociceptors from inflamed skin. Both the antinociceptive effects and the decrease in mechanically evoked responses produced by methAEA and ACEA were blocked by the CB_1 receptor antagonist AM251, strongly suggesting that activation of peripheral CB_1 receptors underlies these effects. Administration of neither methAEA nor ACEA produced antinociception to mechanical stimuli in control, non-inflamed rats nor did it decrease mechanically evoked responses of Aδ nociceptors from non-inflamed skin. These data suggest that attenuation of evoked-responses of Aδ nociceptors contributes to the antiallodynia/antihyperalgesia produced by activation of peripheral CB_1 receptors during inflammation.

Cannabinoid attenuation of allodynia and hyperalgesia

The results of our behavioral studies agree with prior studies demonstrating antiallodynia/antihyperalgesia after local administration of cannabinoids into inflamed tissue through activation of peripheral CB_1 receptors (Amaya et al. 2006; Gutierrez et al. 2007; Richardson et al. 1998). Local administration of cannabinoids have also been shown to attenuate hyperalgesia produced by nerve injury (Fox et al. 2001; Guindon and Beaulieu 2006), cutaneous heat injury (Johanek and Simone 2004), and cancer (Guerrero et al. 2008; Potenzieri et al. 2008) through activation of peripheral CB_1 receptors.

Previous studies have demonstrated that ACEA (Hillard et al. 1999; Meybohm et al. 2008) and methAEA (Abadji et al. 1994) given systemically produced typical cannabinemic effects such as hypothermia, hypolocomotion, catalepsy, and antinociception through activation of CB_1 receptors in the CNS. The doses required to produce these cannabinemic effects were 3- to 10-fold greater than the highest doses of ACEA and methAEA used in the present study (Abadji et al. 1994; Hillard et al. 1999; Meybohm et al. 2008). No antiallodynia or antihyperalgesia occurred when the doses of cannabinoids used in the

present study were injected into the paw contralateral to the inflamed paw, demonstrating that the antihyperalgesic effects of cannabinoids occurred through peripheral mechanisms. The antiallodynia and antihyperalgesia produced by ACEA and methAEA were blocked by co-administration with the CB_1 receptor antagonist AM251 but not with the CB_2 receptor antagonist AM630. The contribution of CB_1 receptors to the antiallodynia/antihyperalgesia produced by ACEA and methAEA is consistent with their higher affinity for CB_1 over CB_2 receptors, 1,400- and 40-fold, respectively (Abadji et al. 1994; Hillard et al. 1999).

We found that intraplantar administration of ACEA and methAEA produced antinociception to mechanical stimuli only during inflammation. Similarly, the ability of ACEA to attenuate behavioral responses to radiant heat was greater during inflammation (Amaya et al. 2006). This enhancement of ACEA's antinociceptive effects was related to increased CB_1 receptor labeling in both nociceptive DRG neurons and their peripheral nerve terminals (Amaya et al. 2006). Similarly, upregulation of CB_1 receptors also occurred in DRG neurons 2 wk after spinal nerve ligation (SNL) (Mitrirattanakul et al. 2006) and was related to the enhanced antinociception produced by locally administered cannabinoids in this model of neuropathic pain (Fox et al. 2001). These studies suggest that peripherally mediated antinociception produced by locally administered cannabinoids results from increased CB_1 receptor expression on nociceptive DRG neurons and presumably their peripheral endings; however, the specific nociceptor subtypes involved have not been determined. It is possible that acute changes also exist to regulate CB_1 receptor activity because locally administered cannabinoids also produce peripherally mediated antinociception in animal models of acute pain such as intraplantar injection of capsaicin (Johanek et al. 2001), cutaneous heat injury (Johanek and Simone 2004), and intraplantar injection of formalin (Calignano et al. 1998; Guindon et al. 2006) through activation of peripheral CB_1 receptors.

We found that administration of CB_1 receptor antagonist AM251 or CB_2 receptor antagonist AM630 alone did not alter mechanical allodynia or hyperalgesia after inflammation evoked by CFA. These results suggest that there were no changes in endocannabinoid tone after inflammation produced by CFA. A similar observation was noted by Gutierrez et al. (2007) after inflammation evoked by carrageenan and using the CB_1 receptor antagonist SR141716A or the CB_2 receptor antagonist SR144528. Additional studies are needed to delineate the potential role of endocannabinoids during inflammation and their relevance to changes in hyperalgesia.

Sensitization of nociceptors during inflammation

Under pathological conditions, nociceptors can become sensitized, which is characterized by a decrease in response threshold, increased responses to suprathreshold stimuli, and ongoing activity (Bessou and Perl 1969; for review, see Raja et al. 1988; Treede et al. 1992). Nociceptor sensitization has been shown to correlate with psychophysical measures of hyperalgesia in humans (LaMotte et al. 1982, 1983; Meyer and Campbell 1981; Torebjörk et al. 1984). Previous studies have demonstrated that cutaneous Aδ and C nociceptors innervating glabrous skin are sensitized after intraplantar injection of CFA both in vivo (Andrew and Greenspan 1999; Djouhri et al. 2006) and in ex vivo preparations (Du et al. 2003, 2006; Wenk et al. 2006). Other studies of inflammatory pain using carrageenan have also demonstrated that Aδ and C nociceptors innervating non-glabrous skin exhibit enhanced responses to natural stimuli (Kirchhoff et al. 1990; Kocher et al. 1987; Koltzenburg et al. 1999).

We found that Aδ nociceptors innervating inflamed skin exhibited ongoing activity, a decrease in mechanical response thresholds, and enhanced responses to suprathreshold mechanical stimuli. Although we were unable to study Aδ nociceptors before and after the induction of inflammation, the functional classification of units in our study allowed

for meaningful comparisons between Aδ nociceptors from non-inflamed and inflamed skin. Similar findings were reported by Andrew and Greenspan (1999) with the exception that they did not observe decreases in mechanical response thresholds, perhaps due to differences in sample size (40 vs. 145 in the present study). Similar to that study, we found no heat-responsive Aδ nociceptors from non-inflamed skin, whereas a small proportion of Aδ nociceptors from inflamed skin were sensitive to heat, as also found by Wenk et al. (2006). However, a higher proportion of heat-responsive Aδ nociceptors were reported innervating the plantar surface of the hindpaw of naive rats as compared with our sample (Leem et al. 1993). The low proportion of heat-responsive Aδ nociceptors in our study was likely due to heat stimuli used (51°C for 5 s vs. 52°C for 20 s) that would have excluded Aδ nociceptors with higher heat response thresholds (Leem et al. 1993).

Contributions of Aδ nociceptors to mechanical allodynia and hyperalgesia

Consistent with hyperalgesia observed 24 h after intraplantar injection of CFA, responses of Aδ nociceptors from inflamed skin evoked by the 26 g von Frey filament, the same filament used to characterize hyperalgesia in our behavioral studies, were enhanced compared with responses of Aδ nociceptors from non-inflamed skin. This enhanced responsiveness of Aδ nociceptors from inflamed skin suggests a contribution of Aδ nociceptors to mechanical hyperalgesia produced by CFA. Mechanical response thresholds of Aδ nociceptors from inflamed skin were lower than thresholds of Aδ nociceptors from non-inflamed skin; however, their respective thresholds were still below paw withdrawal thresholds in behavioral studies of non-inflamed and inflamed rats. The contribution of Aδ nociceptors to mechanical allodynia after intraplantar injection of CFA likely resides in their enhanced evoked responses rather than changes in thresholds. In a similar study after an incision-injury to the plantar surface of the rat

hindpaw, decreased mechanical response thresholds and increased responses to suprathreshold stimuli were also correlated to decreases in paw withdrawal thresholds and increases in paw withdrawal frequencies (Hämäläinen et al. 2002; Pogatzki et al. 2002).

Responses of Aδ nociceptors to intraplantar injection

Injection of cannabinoids or vehicles into mechanical RFs of Aδ nociceptors from both non-inflamed and inflamed skin produced excitation during the injection, termed injection responses. There were no within-group differences of injection responses exhibited by Aδ nociceptors regardless of the cannabinoid or vehicle injected. These results suggest that injection responses are a non-specific effect, possibly due to mechanical distention within RFs (Hilliges et al. 2002). Responses during injections were greater in magnitude in Aδ nociceptors from inflamed skin than the injection responses of Aδ nociceptors from non-inflamed skin. This overall increase in the response during the injection is likely related to the enhanced sensitivity to mechanical stimulation during inflammation.

Injection of cannabinoid or vehicle into mechanical RFs of both Aδ nociceptors from non-inflamed and inflamed skin produced excitation that persisted after injection, termed post-injection responses. A greater proportion Aδ nociceptors from inflamed skin exhibited post-injection impulses compared with Aδ nociceptors from non-inflamed skin. Post-injection responses of Aδ nociceptors from inflamed skin did not differ in proportion and magnitude regardless of the cannabinoid or vehicle injected, also suggesting a nonspecific effect and probably reflects enhanced sensitivity to mechanical stimulation.

Cannabinoid modulation of Aδ nociceptors

After injection of either ACEA or methAEA into the RFs of Aδ nociceptors from inflamed skin, mechanically evoked responses were attenuated and returned to baseline levels by 60 min after injection.

This is similar to the behavioral time course of antiallodynia/antihyperalgesia after injection of ACEA or methAEA in our behavioral studies. Additionally, administration of the CB_1 receptor antagonist AM251 attenuated both the decrease in mechanically evoked responses and antiallodynia/antihyperalgesia produced by ACEA and methAEA, strongly suggesting that activation of peripheral CB_1 receptors underlies these effects. We did not administer the CB_2 receptor antagonist AM630 in the electrophysiological studies because AM630 did not alter the antihyperalgesic/antiallodynic effects of ACEA and methAEA in behavioral studies. Surprisingly, injection of cannabinoids or vehicles did not alter mechanical response thresholds of Aδ nociceptors from inflamed skin at any time point tested. This lack of change in mechanical response thresholds likely reflects the greater contributions of the magnitude of evoked responses in mediating changes to mechanical sensitivity.

The decrease in mechanically evoked responses after administration of ACEA and methAEA was likely due in part to direct activation of CB_1 receptors located on Aδ nociceptors. Previous studies using immunohistochemical methods have localized CB_1 receptors to DRG neurons with nociceptive phenotypes that have either myelinated or unmyelinated fibers, indicating that at least a proportion of both Aδ and C nociceptors express CB_1 receptors (Agarwal et al. 2007; Amaya et al. 2006; Bridges et al. 2003; Khasabova et al. 2002). Although we focused specifically on Aδ nociceptors, the effects of cannabinoids on response properties of C nociceptors need to be determined. We cannot rule out potential actions of ACEA and methAEA on other cell types in the cutaneous environment, which could potentially affect nociceptive sensitivity and nociceptor activity. Fibroblasts (Ständer et al. 2005), endothelial cells (Liu et al. 2000), lymphocytes (Parolaro 1999), mast cells (Samson et al. 2003), keratinocytes (Maccarrone et al. 2003), T-cells (Maccarrone et al. 2001), and dendritic cells (Matias et al. 2002) all express CB_1 receptors.

We did not find any evidence of CB_2 receptor-mediated antinociceptive effects in our study because the CB_2 receptor antagonist AM630 did not block the antinociceptive effects produced by ACEA and methAEA. This was likely due to the higher selectivity of ACEA and methAEA for CB_1 receptors over CB_2 receptors (1,400- and 40-fold, respectively). However, previous studies using selective CB_2 receptor agonists have demonstrated that these drugs produce antinociception to noxious heat (Ibrahim et al. 2005, 2006; Malan et al. 2001) and in a variety of pain models including hyperalgesia produced by carrageenan (Elmes et al. 2005; Gutierrez et al. 2007; Nackley et al. 2003; Quartilho et al. 2003), capsaicin (Hohmann et al. 2004), and neuropathic pain (Ibrahim et al. 2003). Locally administered CB_2 receptor agonists have also been shown to decrease evoked responses of nociceptive spinal cord neurons through activation of peripheral CB_2 receptors (Elmes et al. 2004; Nackley et al. 2004; Sokal et al. 2003). Although CB_2 receptors are mainly expressed on leukocytes, studies have demonstrated that nociceptive DRG neurons express functional CB_2 receptors (Anand et al. 2008; Sagar et al. 2005). Further studies are needed to determine how selective activation of peripheral CB_2 receptors affects the excitability and response properties of nociceptors.

In contrast to the effects on Aδ nociceptors from inflamed skin, administration of methAEA or ACEA transiently increased mechanically evoked responses of non-inflamed Aδ nociceptors. This small increase in evoked responses was related to decreased paw withdrawal thresholds and a trend for an increase in paw withdrawal frequencies in behavioral studies after intraplantar injection of either cannabinoid into non-inflamed hindpaws. This disparity between decreased paw withdrawal thresholds and a trend for an increase in paw withdrawal frequencies suggests that paw withdrawal threshold may be a more sensitive measure than withdrawal frequency using the 26 g von Frey monofilament. The enhanced responses of Aδ nociceptors and

increased mechanical sensitivity in behavioral studies likely resulted from irritation produced by the injection.

Summary

Local administration of cannabinoids, ACEA or methAEA, into inflamed hindpaws attenuated mechanical allodynia and hyperalgesia through activation of peripheral CB_1 receptors. In parallel studies, we found that local administration of either ACEA or methAEA decreased mechanically evoked responses of Aδ nociceptors from inflamed skin that was also attributed to activation of CB_1 receptors. Overall these results suggest that attenuation of Aδ nociceptors' responses contributes to the antiallodynia/antihyperalgesia produced by activation of peripheral CB_1 receptors after local administration of ACEA and methAEA during inflammation. Taken together, our data suggest that peripherally acting cannabinoids could be a potential therapeutic treatment for chronic inflammatory pain.

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Footnotes

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SECTION G ATTACHMENT

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SECTION H

This research document illustrates the clinical relevance and medical benefits derived by the therapeutic efficacy of cannabis which thereby warrants including Psoriasis and Psoriatic Arthritis to the List of chronic Debilitating Conditions for treatment under the Connecticut Medical Marijuana Program.

Cannabinoid receptors (CB) are expressed throughout human skin epithelium. CB1 activation inhibits human hair growth and decreases proliferation of epidermal keratinocytes. Since psoriasis is a chronic hyperproliferative, inflammatory skin disease, the therapeutic modulation of CB signaling, which can inhibit both proliferation and inflammation.

Zakany, Nora; Toth, Balazs I.; Biro, Tamas

NOTE: Cannabinoid receptors are activated by three major groups of ligands, endocannabinoids (produced by the mammalian body), plant cannabinoids (such as THC, produced by the cannabis plant) and synthetic cannabinoids (such as HU-210). All of the endocannabinoids and plant cannabinoids are lipophilic, i.e. fat soluble, compounds.

A novel control of human keratin expression: cannabinoid receptor 1-mediated signaling down-regulates the expression of keratins K6 and K16 in human keratinocytes *in vitro* and *in situ*

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Abstract

Cannabinoid receptors (CB) are expressed throughout human skin epithelium. CB1 activation inhibits human hair growth and decreases proliferation of epidermal keratinocytes. Since psoriasis is a chronic hyperproliferative, inflammatory skin disease, it is conceivable that the therapeutic modulation of CB signaling, which can inhibit both proliferation and inflammation, could win a place in future psoriasis management. Given that psoriasis is characterized by up-regulation of keratins K6 and K16, we have investigated whether CB1 stimulation modulates their expression in human epidermis. Treatment of organ-cultured human skin with the CB1-specific agonist, arachidonoyl-chloro-ethanolamide (ACEA), decreased K6 and K16 staining intensity *in situ*. At the gene and protein levels, ACEA also decreased K6 expression of cultured HaCaT keratinocytes, which show some similarities to psoriatic keratinocytes. These effects were partly antagonized by the CB1-specific antagonist, AM251. While CB1-mediated signaling also significantly inhibited human epidermal keratinocyte proliferation *in situ*, as shown by K6/Ki-67-double immunofluorescence, the inhibitory effect of ACEA on K6 expression *in situ* was independent of its anti-proliferative effect. Given recent appreciation of the role of K6 as a functionally important protein that regulates epithelial wound healing in mice, it is conceivable that the novel CB1-mediated regulation of keratin 6/16 revealed here also is relevant to wound healing. Taken together, our results suggest that cannabinoids and their receptors constitute a novel, clinically relevant control element of human K6 and K16 expression.

Cite this as

Ramot Y, Sugawara K, Zákány N, Tóth BI, Bíró T et al. (2013) A novel control of human keratin expression: cannabinoid receptor 1-mediated signaling down-regulates the expression of keratins K6 and K16 in human keratinocytes *in vitro* and *in situ*. PeerJ 1:e40 http://dx.doi.org/10.7717/peerj.40

Main article text

Introduction

Endocannabinoids as well as exocannabinoids (such as the active components of cannabis) control the function of various types of cells *via* cannabinoid receptor (CB)-dependent or independent manner (Kupczyk, Reich & Szepietowski, 2009). The endocannabinoid system (ECS) consists of these CBs, their endogenous ligands (i.e. endocannabinoids, such as anandamide [AEA] and 2-arachidonoylglycerol), and enzymes responsible for endocannabinoid synthesis and degradation (Biro et al., 2009). In human skin, many different types of cells are now known to express functional CBs (Biro et al., 2009; Czifra et al., 2012; Pucci et al., 2012; Roelandt et al., 2012; Stander et al., 2005; Sugawara et al., 2012; Telek et al., 2007; Toth et al., 2011). The ECS is increasingly appreciated as an important regulator of skin function in

health and disease. For example, the ECS has become implicated in pain (Khasabova et al., 2012; Walker & Hohmann, 2005) and itch control (Stander, Reinhardt & Luger, 2006), and the modulation of inflammation (Klein, 2005) and allergy (Karsak et al., 2007). In addition, CB1 signaling is important in mast cell activation and intracutaneous mast cell maturation from resident progenitors (Sugawara et al., 2012). Furthermore, it regulates fibrosis (Akhmetshina et al., 2009), sebocyte differentiation (Dobrosi et al., 2008) and eccrine epithelial biology (Czifra et al., 2012). Nevertheless, the functions of CB-mediated signaling in human keratinocytes (KCs) *in situ* are as yet poorly understood.

We have previously shown that outer root sheath KCs of human hair follicles (HFs) express CB1. CB1 stimulation by the endocannabinoid, AEA, markedly inhibited human HF growth by inhibiting hair matrix KC proliferation and inducing apoptosis, thus leading to premature HF involution (catagen development). This was reversed by the CB1-specific antagonist, AM251 (Telek et al., 2007). Similarly, human epidermal KC express CBs, and their differentiation is regulated *via* CB1 (Maccarrone et al., 2003; Paradisi et al., 2008). AEA also markedly suppresses human epidermal KC proliferation and induces apoptosis *via* CB1 *in vitro* and *in situ* (Toth et al., 2011). This suggests that the ECS could become a useful therapeutic target in the management of chronic hyperproliferative human skin diseases, such as psoriasis (Toth et al., 2011).

However, it remains unclear whether and how CB1-mediated signaling impacts on human KC differentiation, namely on the expression of hyperproliferation-associated keratins. Psoriasis is characterized by the upregulation of keratins K6 and K16 expression within lesional epidermis (Korver et al., 2006; Mommers et al., 2000). This pair of keratins is also prominently up-regulated in the epidermis under wound healing conditions in men and mice (Moll, Divo & Langbein, 2008; Paladini et al., 1996; Rotty & Coulombe, 2012) and is constitutively expressed in the outer root sheath KCs of human HFs (Langbein & Schweizer, 2005; Moll, Divo & Langbein, 2008; Ramot et al., 2009). Psoriasis is a chronic inflammatory, hyperproliferative dermatosis that, in addition to its anti-proliferative properties (Telek et al., 2007; Van Dross et al., 2012), might also profit from the well-recognized anti-inflammatory properties of CB1-mediated signaling (Sugawara et al., 2012; Wilkinson & Williamson, 2007). Therefore, we have investigated whether CB1 stimulation modulates K6 and K16 expression in human skin. This question was made particularly interesting in view of the most recent discovery that, in murine skin, K6 is not only a wound healing-associated keratin, but actively down-regulates KC migration during wound repair (Rotty & Coulombe, 2012).

In order to answer this question, we used the CB1-specific agonist, arachidonoyl-chloro-ethanolamide (ACEA) (Harvey et al., 2012), and checked its effect on K6 expression *in situ*. This was done by utilizing full thickness human skin organ culture (Lu et al., 2007) as a physiologically and clinically relevant model to study multiple aspects of human skin biology under clinically relevant conditions *in vitro* (Bodo et al., 2010; Knuever et al., 2012; Langan et al., 2010; Lu et al., 2007; Sugawara et al., 2012). In order to confirm the CB1-specificity of the observed effects of ACEA, we also used the CB1-specific antagonist, AM251 (Chanda et al., 2011).

K16 serves as the type I keratin partner of K6 in the formation of intermediate filament heterodimers (Moll, Divo & Langbein, 2008). It is also involved in epidermal barrier function (Grzanka et al., 2012; Thakoersing et al., 2012), and is up-regulated in hyperprolifeative conditions of the skin such as psoriasis (Iizuka et al., 2004) and atopic dermatitis (Grzanka et al., 2012). Therefore, we also examined the effects of ACEA on K16 expression.

HaCaT cells are a highly proliferating human KC line known to overexpress K6 (Ryle et al., 1989). Since HaCaT KCs share some other characteristics with psoriatic KCs and are often employed as surrogate

"psoriatic" KCs (Balato et al., 2012; Farkas et al., 2001; George et al., 2010; Kim et al., 2011; Ronpirin & Tencomnao, 2012; Saelee, Thongrakard & Tencomnao, 2011), we also tested whether and how ACEA modulated K6 expression in these cells *in vitro*. In order to delineate whether any such effects on keratin expression resulted only indirectly from a possible down-regulation of KC proliferation by CB1 stimulation (Toth et al., 2011), double-labeling and quantitative immunohistomorphometry for both K6 and Ki-67 was performed. Finally, to investigate whether K6-expressing human KCs co-express CB1, double-immunolabeling for both antigens was employed.

Materials and methods

Human skin organ culture

Isolated human skin samples obtained from elective plastic surgery procedures (32 pieces of skin fragments obtained by 4 mm punch biopsies from 4 individuals; 3 females and a male aged 26–74, average: 56.5; 3 skin samples were taken from the scalp and one was taken from the hip) were maintained in supplemented serum-free William's E medium as previously reported (Bodo et al., 2010; Knuever et al., 2012; Lu et al., 2007; Poeggeler et al., 2010). Human tissue collection and handling was performed according to Helsinki guidelines, after institutional review board ethics approval (University of Luebeck) and informed patient consent.

Skin samples were first incubated overnight to adapt to culture conditions after which the medium was replaced and vehicle or test substances were added. For human skin organ culture, skin samples were treated with ACEA (Sigma-Aldrich, Taufkirchen, Germany, 30 μM) or AM251 (Sigma-Aldrich, 1 μM), or the combination of them for 1-day after the overnight incubation (Sugawara et al., 2012). Following culturing for the time indicated, skin samples were cryoembedded and prepared for histology, immunohistochemistry/immunofluorescence and quantitative immunohistomorphometry (Ramot et al., 2010; Ramot et al., 2011; Sugawara et al., 2012). Each evaluation was performed on 2–4 sections of 2 skin fragments per each treatment group from 2–4 individuals.

Cell culture

Human immortalized HaCaT KCs (Boukamp et al., 1988) were cultured in DMEM (Sigma-Aldrich) supplemented with 10% fetal bovine serum (Invitrogen, Paisley, UK) and antibiotics (PAA Laboratories, Pasching, Austria). For qRT-PCR, the cells were cultured with ACEA (1 μM) for 8 h.

qRT-PCR

qRT-PCR was performed on an ABI Prism 7000 sequence detection system (Applied Biosystems/Life Technologies, Foster City, CA, USA) using the 5' nuclease assay as detailed in our previous reports (Toth et al., 2011; Toth et al., 2009). Total RNA was isolated from HaCaT keratinocytes using TRIreagent (Applied Biosystems/Life Technologies, Foster City, CA, USA) and digested with recombinant RNase-free DNase-1 (Applied Biosystems) according to the manufacturer's protocol. After isolation, one μg of total RNA was reverse-transcribed into cDNA by using High Capacity cDNA kit (Applied Biosystems) following the manufacturer's protocol. PCR amplification was performed by using specific TaqMan primer and probes (Applied Biosystems, assay ID: Hs01699178_g1 for human K6A). As internal housekeeping gene control, transcripts of cyclophillin A (PPIA) were determined (Assay ID: Hs99999904 for human PPIA). The amount of the K6A transcripts was normalized to the control gene using the ΔCT method.

Immunohistochemistry

For the detection of K6 in organ cultured human skin as well as cultured HaCaT KCs, indirect immunofluorescence staining was performed using mouse anti-human K6 antibody (Progen, Ks6.KA12) at 1:10 dilution as a primary antibody and rhodamine conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories, West Grove, PA) at 1:200 dilution in phosphate-buffered saline (PBS) as a secondary antibody.

To study the proliferation of epidermal KCs, double-immunostaining for K6 and Ki-67 was performed. Briefly, after the staining for K6 with FITC conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories) as a secondary antibody, sections were incubated overnight at 4 °C with a mouse anti-human Ki-67 antibody (DAKO, Hamburg, Germany) at 1:20 in PBS. Sections were then washed with PBS, followed by incubation with rhodamine conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories) (1:200 in PBS, 45 min) at room temperature.

To investigate the localization of CB1 and K6, double immunostaining was performed. For CB1 immunostaining, the highly sensitive tyramide signal amplification (TSA) technique (Perkin Elmer, Boston, MA) was applied. Cryosections were incubated overnight at 4 °C with rabbit anti-CB1 (Santa Cruz, CA, USA) at 1:400 diluted in TNB (Tris, NaOH, Blocking reagent, TSA kit; Perkin-Elmer). Thereafter, the samples were labeled with goat biotinylated antibody against rabbit IgG (Jackson Immunoresearch Laboratories) at 1:200 in TNB for 45 min at room temperature. Sections were then stained with streptavidin-conjugated horseradish peroxidase (1:100, 30 min, TSA kit) and were finally incubated with rhodamine conjugated tyramide (1:50, TSA kit). The TSA method was applied according to the manufacturer's protocol. For the second primary labeling, mouse anti-human K6 antibody (Progen) was applied at 1:20 in PBS, overnight at 4 °C. After the wash with PBS, the sections were incubated with FITC conjugated goat anti-mouse IgG (Jackson Immunoresearch Laboratories) (1:200 in PBS, 45 min) at room temperature.

For K16 antigen detection, we used the LSAB (DCS, Germany) detection method (Ramot et al., 2009) using the K16-gp as primary antibody (guinea-pig, PROGEN, Heidelberg, Germany, dilution 1:1000, GP-CK16), and biotinylated goat anti-guinea pig as secondary antibody (Vector Laboratories, Burlingame, CA, USA, dilution 1:200). HistoGreen (Linaris, Wertheim-Bettingen, Germany) was used as peroxidase substrate.

For all immunostainings, the respective primary antibodies were omitted as negative controls, and morphological criteria and reproduction of the previously published intracutaneous expression patterns of the examined antigens were used as internal positive and negative controls (Moll, Divo & Langbein, 2008). For all experiments, control and treated sections were stained (and later evaluated) on the same day by the same investigator. To avoid staining biases, we calculated the relative staining intensity (arbitrary intensity; 1 as control group) among treatment groups per each individual and then pooled data from all of the experiments.

High magnification images of K6/Ki67 double immunofluorescence and K6 immunofluorescence on HaCaT cells were taken by laser scanning confocal microscopy (Fluoview 300, Olympus Tokyo, Japan) running Fluoview 2.1 software (Olympus).

The staining intensity of K6 and K16 in defined reference areas was assessed by quantitative immunohistomorphometry using the ImageJ software (NIH: National Institutes of Health, Bethesda, MD) (Bodo et al., 2010; Ramot et al., 2010; Ramot et al., 2011). For epidermal evaluation, staining intensity was evaluated in the suprabasal cells, and for HaCaT cells, staining intensity was measured in the colonies formed. For K6 immunofluorescence intensity, skin sections from 4 different individuals were

used, while K16 immunofluorescence intensity and %Ki67 were evaluated in skin sections from 2 patients. 4–6 sections per one individual (2 sections per investigated skin fragment) were used for each evaluation. Each section was evaluated in two/three different non-adjacent microscopic fields (×200), and the mean intensity was measured, and considered as a value. Each treatment group was compared to the control group (average value), and relative change in expression was calculated. Highly comparable results were obtained from different sections from different individuals.

Statistical analysis

Significance of difference between two groups was evaluated using Student's t-test for unpaired samples. For multiple comparisons, one-way analysis of variance (ANOVA) was used, followed by Bonferroni's multiple comparison test, using Prism 5.0 software (GraphPad Prism Program, GraphPad, San Diego, CA). p values <0.05 were regarded as significant. All data in the figures are expressed as mean + SEM. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ for the indicated comparisons.

Results and discussion

The CB1-selective agonist, ACEA, down-regulates K6 protein expression *in situ*

First, we asked whether the CB1-specific synthetic agonist ACEA (Pertwee et al., 2010; Sugawara et al., 2012) can modulate the expression of keratin K6 in human skin. K6 staining intensity within the epidermis of full-thickness human skin that had been organ-cultured for 24 h under serum-free conditions in the presence of ACEA (30 μM) or vehicle alone was assessed by quantitative immunohistomorphometry. This showed that K6 immunoreactivity (IR) was significantly reduced after ACEA treatment, compared to the vehicle control group (Figs. 1A and 1B).

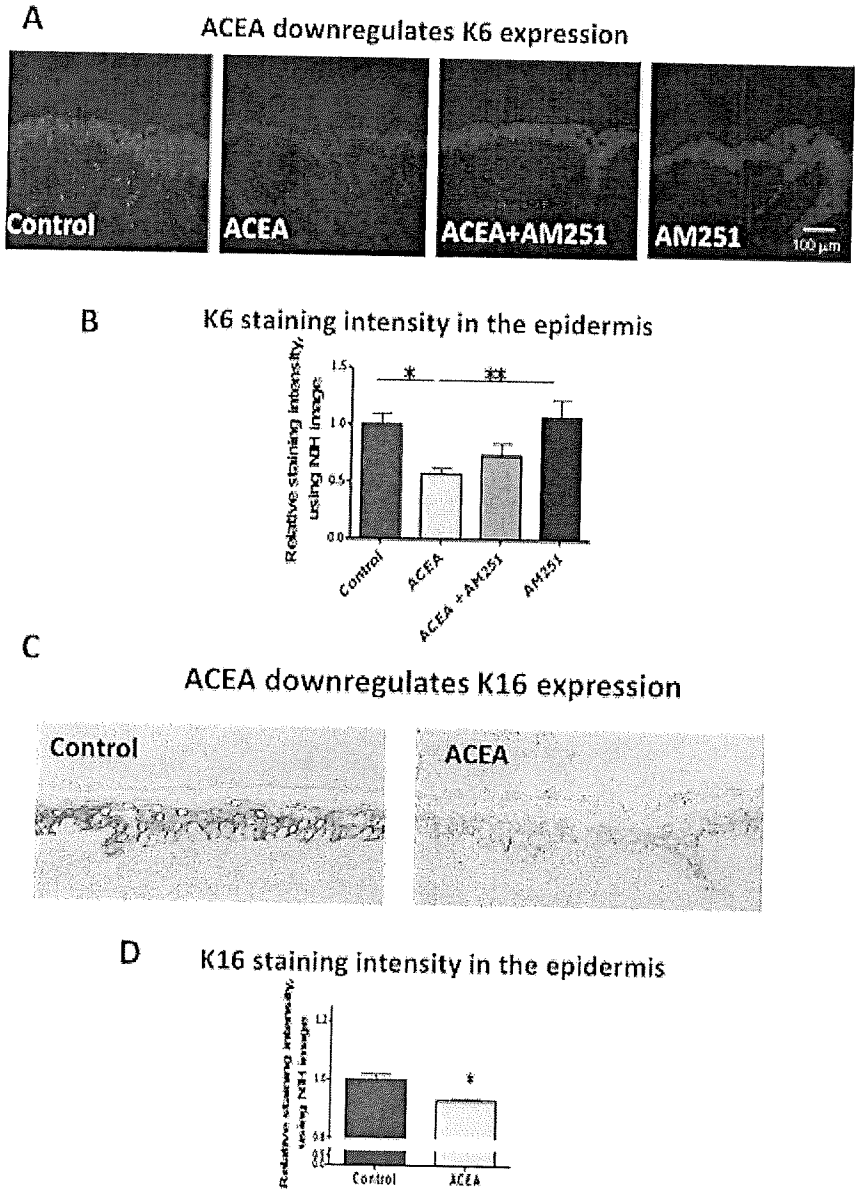

Figure 1: The CB1 specific agonist, ACEA significantly inhibits K6 and K16 expression *in situ*.

(A) Representative images of K6 immunofluorescence with organ cultured human skin treated with ACEA/AM251 (1-day). (B) Statistical analysis of K6 immunofluorescence intensity in organ cultured human skin (quantitative immunohistomorphemtry, ImageJ); stimulation with ACEA (30 µM), AM251 (1 µM) or both for 1-day. n = 9–22 skin sections/group. (C) Representative images of K16 immunohistochemistry with organ cultured human skin samples with ACEA (1-day). (D) Quantitative K16 immunohistomorphometry within the epidermis of organ-cultured human skin samples after 1-day of stimulation with ACEA (30 µM). n = 4 skin sections/group. Data are expressed as mean + SEM. *$p < 0.05$, **$p < 0.01$.

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This down-regulation was abrogated in part by the co-administration with the CB1-specific antagonist, AM251 (Pertwee et al., 2010; Sugawara et al., 2012) (Figs. 1A and 1B). Therefore, intraepidermal K6 protein expression in normal human skin *in situ* is down-regulated in a CB1-specific manner.

ACEA also down-regulates K16 protein expression *in situ*

Since K16 is the type I keratin partner of K6 in KCs and is thought to stabilize this keratin protein as a cytoskeletal heteropolymeric intermediate filament (Ramot et al., 2009), we next analyzed K16 IR in the epidermis of organ cultured human skin samples treated with ACEA. In line with the K6 protein expression, K16 IR was also significantly down-regulated by ACEA *in situ* (Figs. 1C and 1D).

CB1-mediated signaling also regulates K6 expression in cultured, hyperproliferative human keratinocytes

In order to check whether the observed CB1-mediated effects on K6 regulation within intact human skin epithelium depend on intact epithelial-mesenchymal interactions between epidermis and dermis, or are likely to reflect a direct impact of CB1 ligands on epidermal keratinocytes, we next investigated K6 expression in cultured human HaCaT KCs. This transformed human KC line is well-appreciated to constitutively express K6 and to be hyperproliferative (just like human wounded and psoriatic KC) (Balato et al., 2012; Farkas et al., 2001; George et al., 2010; Kim et al., 2011; Ronpirin & Tencomnao, 2012; Ryle et al., 1989; Saelee, Thongrakard & Tencomnao, 2011). K6 is expressed in hyper-proliferative cells (Weiss, Eichner & Sun, 1984) and both K6 expression and basal layer epidermal KC proliferation are increased in psoriasis lesions (Donetti et al., 2012; Griffiths & Barker, 2007; Korver et al., 2006; Litvinov et al., 2011; Mommers et al., 2000). HaCaT cells are known to express functional CB1 and CB2 (Leonti et al., 2010; Maccarrone et al., 2003; Paradisi et al., 2008), and this had been confirmed previously by our group, both on the gene (RT-PCR analysis) and protein levels (immunocytochemistry and western blotting techniques) (Toth et al., 2011). Thus, being a direct target of CB1-mediated signaling, this makes these KCs not only an instructive cell culture tool for evaluating the direct, dermis-independent role of CB1-mediated signaling in the regulation of keratin expression in human KCs, but may also provide first indications as to how the observed K6 expression could relate to wound healing and/or psoriasis.

In accordance with our human skin organ culture results, ACEA (1 μM) significantly down-regulated K6 staining intensity of HaCaT cells *in vitro* (Figs. 2A and 2B). This was abrogated by the co-administration of the selective CB1 antagonist, AM251 (100 nM) (Figs. 2A and 2B). Unexpectedly, though, AM251 alone had a partial inhibitory effect on K6 expression, although not significant. This may be related to the fact that AM251 is an inverse agonist (Dono & Currie, 2012; Fiori et al., 2011), and invites further study. The inhibitory effect of ACEA on K6 protein expression was further confirmed by quantitative RT-PCR (Fig. 2C). Therefore, while HaCaT cells may exhibit relatively low CB expression levels, at least under our assay conditions, they showed a vigorous response to CB ligands.

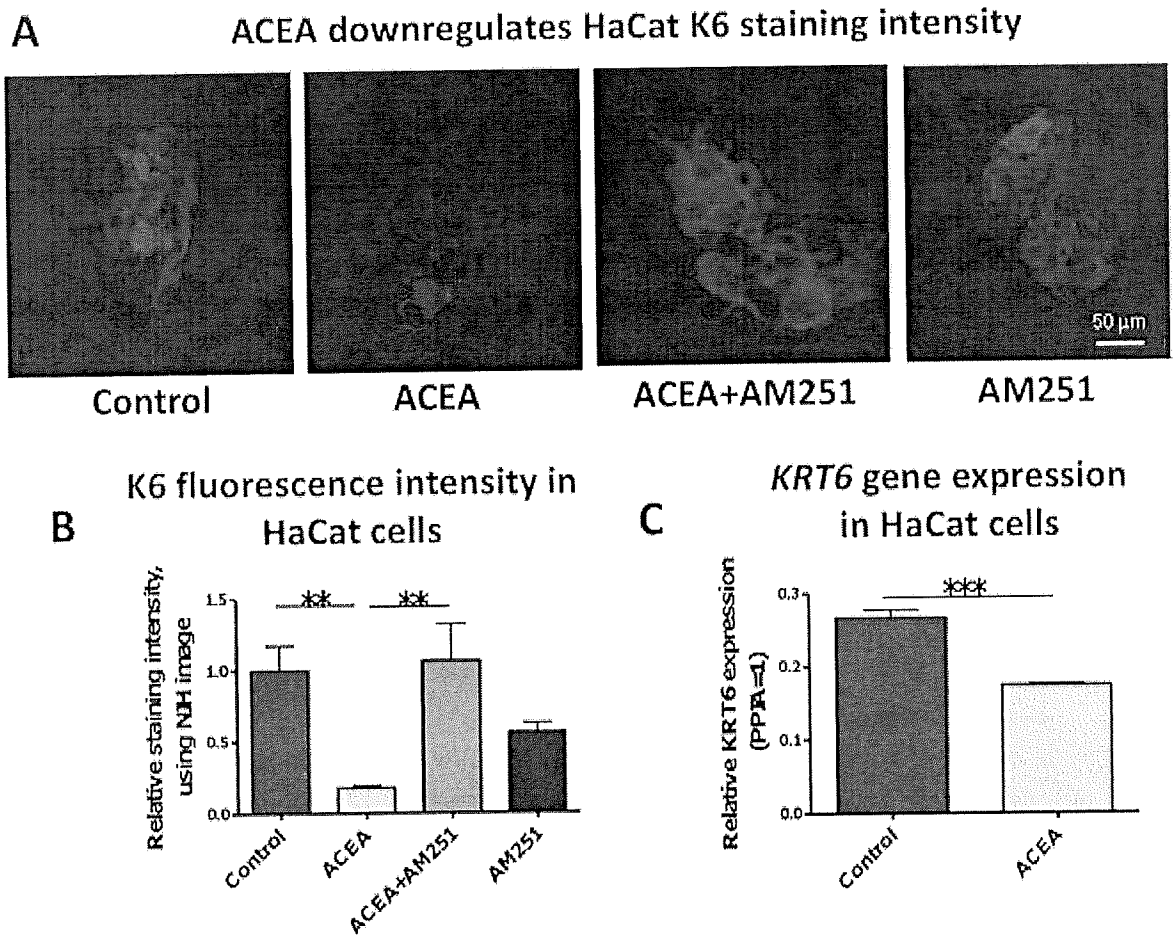

Figure 2: The CB1 specific agonist, ACEA significantly inhibits K6 expression in cultured HaCaT cells.

(A) Representative images of K6 immunofluorescence of cultured HaCaT KCs with ACEA (1 µM), AM251 (100 nM) or both for 1-day. (B) Statistical analysis of K6 immunofluorescence intensity of cultured HaCaT cells. n = 6 colonies/group (C) Statistical analysis of K6 gene expression in HaCaT cells treated with vehicle control or ACEA (1 µM) for 8 h. Data are expressed as mean + SEM. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

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The CB1 specific agonist, ACEA significantly decreases human epidermal keratinocyte proliferation *in situ*

However, the observed effects of CB1-mediated signaling on epidermal K6 expression could simply reflect the appreciated anti-proliferative effects of CB1 agonists (Casanova et al., 2003; Hermanson & Marnett, 2011; Toth et al., 2011). Moreover, K6 is overexpressed in hyper-proliferative and wounded KCs (Weiss, Eichner & Sun, 1984), and both K6 expression and basal KC proliferation are increased in psoriatic epidermal lesions (Donetti et al., 2012; Griffiths & Barker, 2007; Korver et al., 2006; Litvinov et al., 2011; Mommers et al., 2000; Navarro, Casatorres & Jorcano, 1995). Therefore, we next assessed whether CB1 stimulation by CB1 specific agonist, ACEA, could affect human KC proliferation *in situ*.

Just as we had seen before with the non-selective endocannabinoid, AEA (Toth et al., 2011), the CB1-specific synthetic agonist, ACEA indeed significantly decreased human epidermal KC proliferation *in situ*. This effect was abrogated by the CB1-specific antagonist, AM251 (assessed by quantitative Ki-67 immunomorphometry, Figs. 3A and 3B).

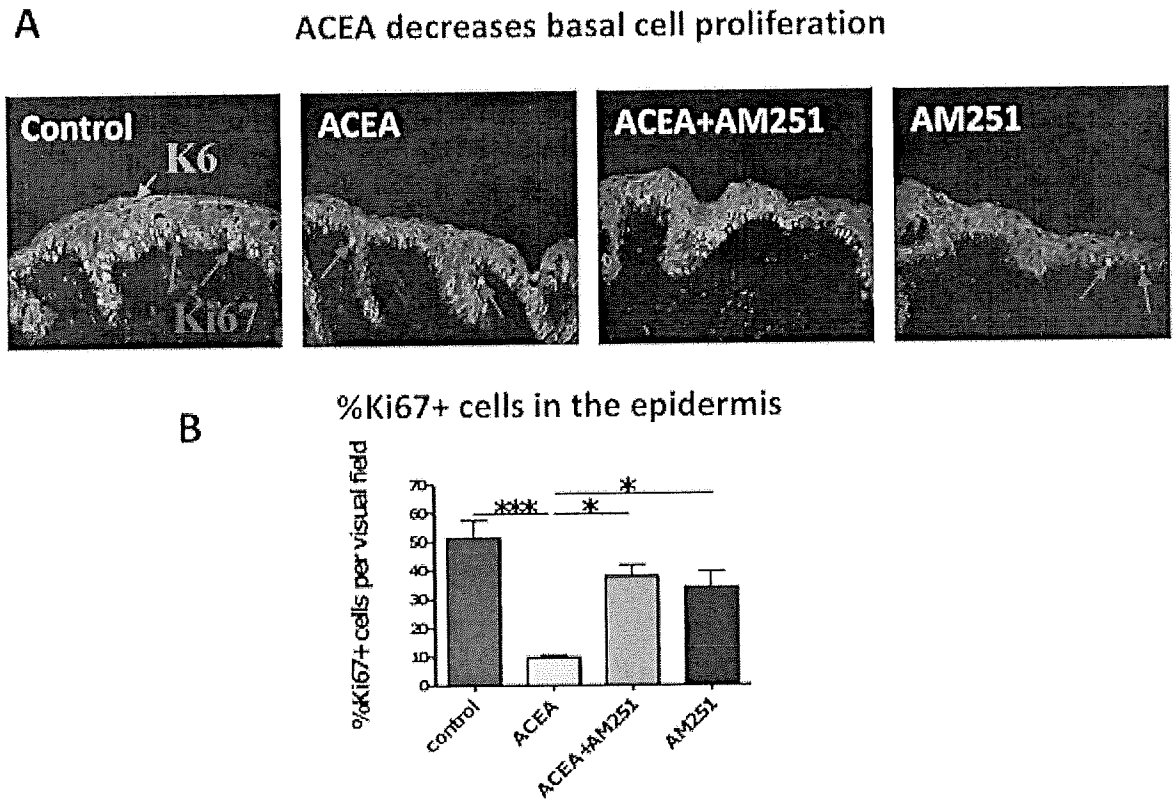

Figure 3: The CB1 specific agonist, ACEA significantly decreases human epidermal keratinocyte proliferation *in situ*.

(A) Representative images of K6 (green) and Ki67 (red) double-immunofluorescence with organ cultured human skin treated with ACEA/AM251 (1-day). (B) Quantitative analysis of the percentage of Ki67 + KCs within organ cultured human epidermis. *$p < 0.05$; ***$p < 0.001$. n = 5–12 skin sections/group.

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The CB1 specific agonist, ACEA, significantly decreases K6 expression in suprabasal cells in a proliferation-independent manner

Therefore, it needed to be dissected whether or not CB1 also reduces K6 expression in a proliferation-independent manner. This was done by selectively assessing K6 expression in non-proliferative (i.e. Ki-67-negative) epidermal KCs *in situ*. We found that K6 IR within non-proliferative epidermis was also reduced by ACEA (Figs. 4A and 4B). Furthermore, K6-expressing cells in the epidermis co-expressed CB1 *in situ* (Fig. 4C), suggesting a direct effect of CB1-agnosits on K6-expressing human epidermal KCs *in situ*. Thus, CB1 stimulation may affect K6 expression both, by reducing KC proliferation and by down-regulating K6 expression directly *via* CB1 in a proliferation-independent manner.

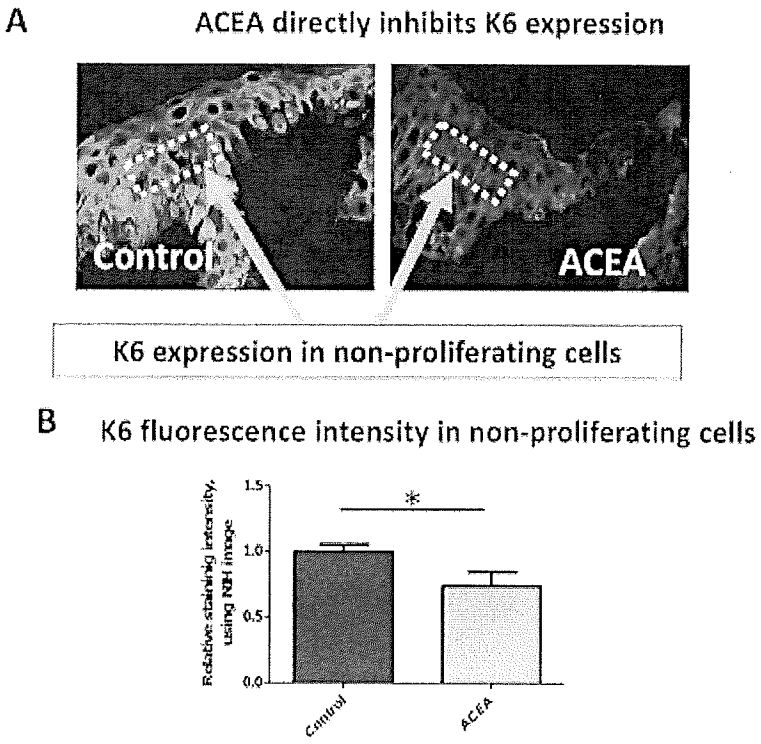

Figure 4: The CB1 specific agonist, ACEA, significantly decreases K6 expression in suprabasal cells in a proliferation-independent manner.

(A) Representative images of K6 (green) and Ki-67 (red) double immunofluorescence. Dotted rectangles indicate the reference area for quantitative immunohistomorphometry of K6 fluorescence intensity. (B) Quantitative analysis of K6 fluorescence intensity in non-proliferating (i.e. Ki67-negative) cells within human epidermis *in situ*. Data are expressed as mean + SEM. $*p < 0.05$. $n = 5–7$ skin sections/group. (C) K6 (green) and CB1 (red) double-immunofluorescence study. Yellow arrows denote double-positive KCs.

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Here we provide the first evidence that CB1-mediated signaling directly regulates K6/16 expression within normal human skin. Specifically, we show that CB1 stimulation down-regulates expression of the

hyper-proliferation-associated human keratins K6 *in vitro* and *in situ*, and inhibits human epidermal KC proliferation *in situ*.

The effect of CB-mediated signaling in human KC biology remains to be fully explored. As we have also observed in isolated human skin (Fig. 4C), CB1 protein expression is detected mainly above the basal layer of the epidermis (Stander et al., 2005), i.e. above the compartment where KC proliferation normally occurs most prominently. Wilkinson and Williamson reported that the non-selective CB agonist HU210 inhibited KC proliferation. However, this could not be blocked by either CB1 or CB2 antagonists, suggesting that cannabinoids may also inhibit human KC proliferation through a non-CB1/CB2 mechanism (Wilkinson & Williamson, 2007). Nevertheless, it has been previously shown that AEA, which can interact with CB1 on human KC (Biro et al., 2009), inhibited human KC proliferation *in situ* and *in vitro* (Toth et al., 2011).

Therefore, it was important to clarify whether specific CB1 stimulation inhibits human epidermal KC proliferation *in situ*. By using CB1-specific agonists and antagonists we clearly demonstrate that exclusive CB1 stimulation inhibited KC proliferation. Thus, CB1 is an important key regulator of human KC proliferation. Given the role of epidermal hyperproliferation in the pathobiology of psoriasis (Donetti et al., 2012; Griffiths & Barker, 2007; Korver et al., 2006; Litvinov et al., 2011; Mommers et al., 2000; Navarro, Casatorres & Jorcano, 1995), cannabimimetic agents that activate CB1, therefore, deserve consideration as a novel pharmacological strategy for treating psoriasis.

Furthermore, increased numbers of activated mast cells are often observed in and around lesional psoriatic skin, and increasing evidence suggests that mast cells are functionally important key immunocytes in the pathogenesis of psoriasis (Carvalho, Nilsson & Harvima, 2010; Namazi, 2005; Radosa et al., 2011; Suttle et al., 2012; Toruniowa & Jablonska, 1988). Recently, we have shown that CB1 activation limits excessive mast cell activity and even inhibits mast cell maturation of resident, intracutaneous progenitors (Sugawara et al., 2012). Therefore, besides their anti-proliferative effects on human epidermal KCs, the anti-inflammatory (Richardson, Kilo & Hargreaves, 1998) and mast cell-inhibitory properties of CB1 agonists in human skin (Sugawara et al., 2012) make them a particularly attractive class of agents in future psoriasis management.

It should be noted, that the constitutive level of K6 expression in organ-cultured human skin fragments is considerably higher than normal scalp skin *in vivo*. Presumably, this occurs as a response to tissue dissection and organ culture, which is well-known to elicit an immediate wound healing response in the epithelium. The latter rapidly starts to migrate over the wound edge in an attempt to enwrap the exposed skin mesenchyme (epiboly phenomenon Stenn, 1981; Brown et al., 1991). This constitutive up-regulation of K6 in organ-cultured normal human skin may greatly heighten the sensitivity to K6 expression-modulatory compounds, such as CB ligands, thus making human skin organ culture a particularly sensitive and instructive tool for clinically relevant keratin research. At the same time, however, caution is advised in extrapolating from these observation made in what essentially reflects a wound healing milieu to healthy, unmanipulated human skin *in vivo*.

The current findings invite the speculation that the therapeutic down-modulation of K6 and/or K16 expression by CB1 agonists and other cannabimimetics might become exploitable for the management of other dermatoses besides psoriasis, for example pachyonychia congenita (Hickerson et al., 2011; Zhao et al., 2011) and acne (Biro et al., 2009), and could be used to modulate KC migration-dependent reepithelialization in wound healing, similar to related findings in periodontal and intestinal wound repair (Kozono et al., 2010; Wright et al., 2005).

Conclusion

Our results suggest that cannabinoids and their receptors constitute a novel, clinically relevant control element of human K6 and K16 expression. Therefore, cannabimimetic agents might be relevant for the treatment of several skin conditions related to aberrant K6/K16 expression, such as psoriasis and wound healing. In addition, skin organ culture is shown to be a clinically and physiologically relevant model system for investigating the effect of CB1 specific agonists/antagonists on human skin.

Abbreviations

ACEA

arachidonoyl-chloro-ethanolamide

AEA

anandamide

CB1

cannabinoid receptor 1

ECS

endocannabinoid system

HF

hair follicle

KC

keratinocyte

Additional Information and Declarations

Competing Interests

Ralf Paus is an Academic Editor for PeerJ.

Author Contributions

Yuval Ramot and Koji Sugawara conceived and designed the experiments, performed the experiments, analyzed the data, wrote the paper.

Nóra Zákány and Balázs I. Tóth performed some of the experiments, and contributed and analyzed qRT-PCR data.

Tamás Bíró contributed and analyzed qRT-PCR data and edited the paper.

Ralf Paus conceived, designed, and supervised the experiments, and wrote the paper.

Human Ethics

The following information was supplied relating to ethical approvals (i.e. approving body and any reference numbers):

Institutional review board ethics committee of the University of Luebeck, reference number 06-109.

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24

Attached is a little known fact that the U.S. Government represented by the Department of Health and Human Services was awarded U.S. Patent Number **6,630,507 Cannabinoids as antioxidants and neuroprotectants. The abstract states Cannabinoids have been found to have antioxidant properties, unrelated to NMDA receptor antagonism. This new found property makes cannabinoids useful in** the treatment and prophylaxis of wide variety of oxidation associated diseases, such as ischemic, age-related, **inflammatory and autoimmune diseases. Psoriasis and Psoriatic Arthritis are of course inflammatory and autoimmune diseases**

(12) **United States Patent**
Hampson et al.

(10) **Patent**
(45) **Date of**

(54) **CANNABINOIDS AS ANTIOXIDANTS AND NEUROPROTECTANTS**

(75) Inventors: **Aidan J. Hampson**, Irvine, CA (US); **Julius Axelrod**, Rockville, MD (US); **Maurizio Grimaldi**, Bethesda, MD (US)

(73) Assignee: **The United States of America as represented by the Department of Health and Human Services**, Washington, DC (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/674,028**

(22) PCT Filed: **Apr. 21, 1999**

(86) PCT No.: **PCT/US99/08769**

§ 371 (c)(1),
(2), (4) Date: **Feb. 2, 2001**

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PCT Pub. Date: **Oct. 28, 1999**

Related U.S. Application Data

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(51) **Int. Cl.**[7] **A61K 31/35**
(52) **U.S. Cl.** **514/454**
(58) **Field of Search** 514/454

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Section J

#25

I have not supplied under this paper work submission a direct letter of support from my physician because he has already done so by already enrolling me in Connecticut's MMP. He was very supportive and eager for me to obtain relief from the severe stress caused by my Psoriasis and psoriatic arthritis; away from protocols that carry the dangers of an increased risk of significant morbidity including skin cancers, lymphoma and liver disease that I find way too frightening. And none of those night mare protocols would help with the agony of the pruritus that cannabis will.

The stress from the symptoms causes the disease to flare and the flare-ups cause the stress. A vicious circle is created which lends itself to post traumatic stress. Post-traumatic stress is recognized as a related possible cause of the disease but it is also a symptom. I am sure under some circumstances that any one of the already approved debilitating conditions could generate a post-traumatic stress condition.

I urge the Board of Physicians to recognize me and others with Psoriasis and psoriatic arthritis as having what is accepted and understood by the medical community at large as an autoimmune, chronic "Debilitating Medical Condition" that qualifies under the State of Connecticut Medical Marijuana Program as it is in other states that have approved medical marijuana.

Sincerely and Respectfully

[redacted]